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Phylogenetic studies of Tribe Cacteae (Cactaceae) with special emphasis on the genus Mammillaria

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Phylogenetic studies of Tribe Cacteae (Cactaceae) with special emphasis on the genus *Mammillaria*

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

General Introduction

Problems in Cactus Classification

The nature of many cacti do not lend themselves to ease of botanical study. Often found growing in inaccessible and inhospitable regions, the study and collection of cacti in the wild is not a trivial matter. Moreover, the succulence of cacti, and the presence of large volumes of mucilage in the plant bodies hinders the preparation of good quality herbarium material. Compounding these problems is the fact that many cacti have incredibly long life-cycles, often taking years to decades before reaching flowering maturity. Furthermore, the lack of typical 'dicotyledonous' leaves also removes a whole suite of characters that are normally associated with taxonomic classification in other plant families.

Historically, the cactus family has been subject to excessive taxonomic splitting and proliferation of names by commercial growers, enthusiastic amateurs and dedicated botanists. Nomenclatural type specimens on occasion have been poor quality herbarium specimens, greenhouse plants, or even photographs. A famous example of poor botanical practice was the description of *Oreocereus crassiniveus*, which was described by Curt Backeberg based on observations from a moving train in Bolivia.

In the preamble of the CITES Cactaceae Checklist (Hunt, 1999), Hunt explains that there are about 6,300 scientific names of cacti in current use. However, Anderson (2001) suggests that since 1753, in excess of 15,000 cacti names have been published. In 1984, the International Organization for Succulent Plant Study (IOS) established a working party to determine whether a consensus on the general classification of the Cactaceae was possible. This working group is still in existence and is currently known as the International Cactaceae Systematics Group.

DNA Studies and the Nested Phylogenetic Approach

Over the last two decades, the use of molecular methods for studying plant phylogenetic relationships has revolutionized our approach to plant systematics. Since the development of PCR (Polymerase Chain Reaction) and direct sequencing of PCR products, the use of sequence data dominates in the production of plant phylogenies. However, it remains prudent to treat cladograms produced from any kind of data as hypotheses of phylogenetic history and not as fact.

To date, there have been relatively few systematic studies of the cactus family utilizing molecular techniques. Such studies include those by Hershkovitz and Zimmer (1997), Cota-Sanchez and Wallace (1997) Cota-Sanchez (1997), Wallace and Dickie (2002), Nyffeler (2002), Porter *et al.* (2000), Wallace (1995) and Wallace and Gibson (2002). However, it is clear that the use of molecular methods provides a good source of data for the study of cactus systematics.

Inherent in any phylogenetic study is the cladistic principle that the direction of evolutionary change for characters needs to be determined. This is usually achieved through *outgroup comparison* whereby data for a number of taxa that lie outside the group of study (ingroup) are obtained in addition to data from the ingroup. However, in many circumstances the choice of suitable outgroups is not trivial, and a study of a larger, more inclusive group may be required to ascertain appropriate outgroups for the original group of study. This approach, known as a *nested phylogenetic approach*, can reveal more precisely membership of groups for further study, while also indicating which outgroup taxa would be most appropriate.

For this dissertation, a *nested phylogenetic approach* was adopted to shed light on the taxonomic complexities of the cactus genus *Mammillaria* Haworth. Previous taxonomic works (Britton and Rose, 1922, 1923; Buxbaum, 1951a, b, 1956a, b; Hunt, 1971, 1977a, b, c, 1981; Lüthy, 1995, 2001; Schumann, 1898) were not consistent in generic delimitations for the genus, and relationships to closely related genera. For this reason, common sense dictated that

a study of Tribe Cacteae (to which *Mammillaria* belongs) would at least allow a number of candidate outgroups to be determined for subsequent use in a more detailed study of *Mammillaria.*

Dissertation Organization

The second chapter of this dissertation, entitled "History of Taxonomic Classification of Tribe Cacteae" summarizes the taxonomic history of tribe Cacteae. The chapter covers the taxonomic placements of members of the tribe Cacteae by early cactus monographers, through to the concept and circumscription of tribe Cacteae as recognized today.

The third chapter, entitled "History of Taxonomic Classification in Mammillaria Haworth" summarizes the taxonomic history of the genus *Mammillaria* from the use of a currently recognized species of *Mammillaria* by Linnaeus as type species for the cactus family through to the most recent taxonomic treatment of the genus. Particular attention is paid to the early phylogenetic hypotheses by Buxbaum (1951a, b, 1954, 1956a, 1956b, 1963), and the modern infrageneric classifications of the genus by Hunt (1979, 1981, 1984, 1986) and Lüthy (1995, 2001).

The fourth chapter, entitled " Molecular systematics of tribe Cacteae (Cactaceae: Cactoideae): a phylogeny based on *rpl*16 intron sequence variation" was published in the journal *Systematic Botany* in 2002. Technical information presented details the protocols and use of sequence data from the intron of the chloroplast *rpl*16 gene for phylogeny reconstruction of members of tribe Cacteae. The resultant phylogeny had a highly pectinate topology and delimited a number of clades that corresponded to previously considered generic groups. A number of morphological series within the tribe were also deduced, including a transition from ribbed stems to tuberculate stems and a transition from flowers produced from tubercle apices to axils. Paraphyly of the genus *Mammillaria* was also demonstrated.

Chapter five, entitled "Systematics and taxonomy of *Mammillaria* (Cactaceae) using non-coding chloroplast DNA sequence variation" is a manuscript prepared for submission to

the journal *Systematic Botany*. This paper utilizes outgroup information ascertained from the previous chapter and dramatically increases sampling from within the genus *Mammillaria*. Chloroplast sequence data from the *psbA-trnH* intergenic spacer and the *rpl*16 intron are used for phylogeny reconstruction using both parsimony and Bayesian techniques. A number of species of *Mammillaria* are discovered to be totally lacking the *rpl*16 intron – a phylogenetically important character. As currently circumscribed, *Mammillaria* is polyphyletic, and shows a basal divergence between those members recognized in series *Ancistracanthae* by Hunt (1981) and the 'core' *Mammillaria* species. The clades revealed from this analysis are further discussed in the context of the infrageneric classifications of Hunt (1981) and Lüthy (1995, 2001).

The sixth chapter, entitled "A localized loss of the chloroplast *rpl*16 intron from *Mammillaria* series *Stylothelae*" is a manuscript to be submitted to the journal Heredity. This chapter details the loss of the *rpl*16 intron from members of the *Crinita* group of *Mammillaria* series *Stylothelae*. Morphological differences are briefly summarized and the distributions of taxa lacking the intron are compared to those in which the intron is present.

Chapter seven presents general conclusions from the studies undertaken in this dissertation. In particular, areas of potential future research are discussed along with work that should be undertaken to strengthen the conclusions of the studies reached in this dissertation.

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CHAPTER 2

History of Taxonomic Classification of Tribe Cacteae

When Linnaeus (1753) described the cactus family it consisted of the single genus *Cactus* L. By 1845, the number of genera had been expanded by subsequent authors to such an extent that Salm-Dyck (1845) divided the family into seven tribes of which two tribes (Melocacteae and Echinocateae) contained members of the currently accepted tribe Cacteae. Charles Lemaire (1868) recognized ten tribes in the family, including a new, although invalidly published tribe 'Les Leuchtenbergiées' to include the recently described species *Leuchtenbergia principis* Hook., which he considered to be intermediate between tribes Melocacteae and Echinocacteae.

In his large, detailed treatment of the Cactaceae, Schumann (1898) presented a classification that included subfamilies Pereskioideae, Opuntioideae and Cactoideae (then known as the Cereoideae). The latter subfamily was divided into three tribes. Many members of the currently circumscribed Cacteae were placed by Schumann into his tribe Mamillarieae. The largely epiphytic genera were grouped into the Rhipsalideae, while the remaining tribe (Echinocacteae) contained a mixed assortment of columnar cacti plus genera that were difficult to place elsewhere. Included in this latter tribe were two genera that are currently recognized as members of tribe Cacteae – *Echinocactus* and *Leuchtenbergia*. Schumann's classification is summarized alongside the classifications of subsequent cactographers in Table 2-1.

Britton and Rose (1919, 1920, 1922, 1923) designed a classification of the Cactaceae that was largely based on the system of Schumann (1898), however, they did not recognize any subfamilies, preferring to relegate Schumann's three subfamilies to the rank of tribe. In this manner, they recognized tribe Cereeae instead of subfamily Cereoideae. Britton and Rose were prolific taxonomic splitters and recognized one hundred and twenty-three genera (as opposed to Schumann's twenty-one), the largest number placed within their tribe Cereeae B. & R., which were distributed among eight subtribes.

Table 2-1. Past tribal classifications in subfamily Cactoideae.

Schumann (1898)	Britton & Rose (1919-1923)	Berger (1926, 1929)	Buxbaum (1958)
			Tribe Leptocereeae
			Tribe Hylocereeae
			Subtribe Nyctocereinae
	Subtribe Cereanae		Subtribe Hylocereinae
	Subtribe Epiphyllanae	Tribe Cereeae, Subtribe Phyllocacteae	Subtribe Epiphyllinae
Group Rhipsalideae	Subtribe Rhipsalidanae	Tribe Rhipsalideae	Subtribe Rhipsalinae
			Tribe Pachycereeae
	Subtribe Cereanae	Tribe Cereeae, Subtribe Cereinae	Tribe Cereeae
			Tribe Trichocereeae
			Subtribe Trichocereinae
			Subtribe Rebutinae
			Subtribe Borzicactinae
	Subtribe Cactanae		Tribe Notocacteae
	Subtribe Echinocereanae		Tribe Echinocereeae
Group Echinocacteae			Tribe Echinocacteae
	Subtribe Echinocactanae	Tribe Cereeae, Subtribe Echinocacteae	Subtribe Echinocactinae
			Subtribe Thelocactinae
Group Mamillarieae		Tribe Cereeae, Subtribe Mamillarieae	Subtribe Ferocactinae
	Subtribe Coryphanthinae		Subtribe Coryphanthinae

With the recognition of eight subtribes in tribe Cereoideae, Britton and Rose were able to segregate members of Schumann's tribe Echinocactae into more discrete groups. The foremost division in Britton and Rose's treatment of tribe Cereeae was that between cacti with monomorphic (spine and flower-bearing) and dimorphic (separate spine and flower-bearing) areoles. This created a major separation in the North American barrel cacti, with subtribe Echinocactinae B. & R. containing those species with monomorphic areoles (the majority of species currently recognized within tribe Cacteae) and species with dimorphic areoles were placed within subtribe Coryphanthinae B. & R.

Although working in a post Britton and Rose environment, Berger (1926, 1929) invoked a Schumannian approach to cactus classification which recognized three subfamilies and presented possible evolutionary scenarios both between and within the subfamilies. Berger (1929) accepted only two tribes within the Cereoideae – Rhipsalideae, and Cereeae Berger. Although Berger only recognized 41 genera in the cactus family, 24 were in tribe Cereeae. For this reason, Berger created three subtribes – Phyllocacteae, Cereinae, Echinocacteae and accepted Schumann's subtribe Mamillarieae. As in previous classifications, the smaller, non-epiphytic barrel cacti were distributed between the Echinocacteae and Mamillarieae, the presence of dimorphic areoles being the key diagnostic feature of the Mamillarieae. The majority of members of tribe Cacteae (as currently circumscribed) were placed into subtribe *Echinocactae*. Anderson (2001) remarks that while Berger made important insights into a natural classification of the Cactaceae, he was also victim of the confusion caused by the numerous genera and species being discovered and described at that period.

In his monumental treatise on the Cactaceae, Backeberg (1958-1962) brought together a classification of the family that he had been formulating since the late 1930's. His system was loosely based upon that of Schumann (1898) in that he recognized three subfamilies. In the Cereoideae, Backeberg followed the system of Berger (1929) which placed members of the Cereoideae into one of two tribes – the Hylocereeae containing the epiphytic cacti while the terrestrial members of the subfamily were placed in tribe Cereeae. Within Cereeae, Backe-

berg created an elaborate system of subtribes, semitribes, groups and subgroups that were based upon the geographic distribution of the cacti and a presumed origin for the family in the region of the Caribbean and Central America. For the first time, a classification system distinguished between members of tribe Cacteae (as currently circumscribed) from their South American counterparts (albeit due to the division of terrestrial cacti in tribe Cereeae, on broad geographical grounds). In this manner, Backeberg's subtribe Boreocactinae begins to approach the circumscription that is currently recognized for tribe Cacteae. As with previous classifications, members of North American barrel cacti were further distinguished based upon the presence of dimorphic areoles, in Backeberg's case, the division was into groups – the Boreoechinocacti Backeberg having undifferentiated areoles and the Mammillariae Berger possessing dimorphic areoles.

A one-time collaborator of Backeberg, Buxbaum developed a natural classification of the cacti that serves as the basis for most current cactus taxonomic systems. Buxbaum focused on studies of flower and seed morphology and utilized this information to produce an evolutionary classification, which followed Schumann (1898) in accepting three cactus subfamilies. Within subfamily Cereoideae, Buxbaum (1958) recognized eight tribes, dismissing the names and system of Backeberg as invalidly published with Latin names that were *nomina nuda*. In this system, Buxbaum amended Schumann's (1898) tribe Echinocacteae to include only the North American barrel cacti, except for the genus *Astrophytum* Lemaire which he placed in tribe Notocacteae Buxbaum on the basis of seed structure.

Within tribe Echinocacteae (= Cacteae), Buxbaum recognized four subtribes, each one representing a major lineage in his phylogeny of the Echinocacteae (Buxbaum, 1951b). Subtribe Echinocactinae B. & R. emend. Buxb. was comprised of *Echinocactus* and *Homalocephala* which were Buxbaum's ancestral members of the tribe, from which all other tribes, subtribes and species were derived. From subtribe Echinocactinae, Buxbaum surmised (based on seed testa morphology) that there had been three main branches of evolution, each one representing a subtribe – Thelocactinae Buxb., Ferocactinae Buxb., and Coryphanthinae Buxb. The

underlying hypothesis for Buxbaum's evolutionary classification was that within tribe Echinocacteae, there had been an evolutionary progression in morphology that iniated with ancestral stage as characterised in subtribe Echinocactinae and lead through four other evolutionary stages until a final '*Mammillaria*-stage' was reached in a number of independent lineages which Buxbaum defined as subtribes (Buxbaum, 1951a, b). This is detailed in Figure 3-3.

To date, there has been only a single attempt at a phylogenetic evaluation of tribe Cacteae since Buxbaum. In his Ph.D. thesis, Zimmerman (1985) undertook a morphological study of the Cacteae as part of his more detailed study on the genus *Coryphantha.* Zimmerman suggested that the Pachycereeae and Notocacteae probably represent the closest outgroup tribes to the Cacteae, going so far as to suggest that the Cacteae had its origins in South America. Although he had misgivings about the placement of *Astrophytum* in the Cacteae, Zimmerman concluded that the tribe likely had a monophyletic origin. The main problem faced by Zimmerman's study was the morphological plasticity that is rampant in the Cacteae, however, he did manage to make some insightful conclusions. Whereas Buxbaum (1951a, b) had hypothesized that members of *Escobaria*, *Ortegocactus*, *Mammillaria* and *Coryphantha* represented the convergent endpoints of more than one line of evolution, Zimmerman concluded that this was unlikely and that these genera were likely derived from a single *Mammillaria*-like ancestor.

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CHAPTER 3

History of Taxonomic Classification in *Mammillaria* Haworth

Following the recent re-organization of the genus *Opuntia* Miller by Wallace and Dickie (2002) into a number of segregate genera, the genus *Mammillaria* Haworth has taken precedence as the most species rich genus in the cactus family. Modern estimates of species numbers vary greatly depending upon circumscription at both the generic and species level. 181 species are recognized by Pilbeam (1999) while Hunt (1999) accepts 145 species.

When Linnaeus developed and published his plant classification system based on binomial names in 1753, he listed only a single genus – *Cactus* L. – in the family Cactaceae. By the end of the eighteenth century, subsequent authors had cleaved a handful of genera from the genus *Cactus.* The most prominent of these workers was Miller, who in the year following Linneaus' Species Plantarum (Linnaeus, 1753) described the genera *Opuntia* and *Pereskia* (Miller, 1754). In 1788, Gaertner described the epiphytic genus *Rhipsalis* bringing the number of genera in the cactus family to four. In 1812, Adrian Hardy Haworth described the genus *Mammillaria* from Linnaeus' genus *Cactus* (Haworth, 1812). However, Haworth was not the first to use the name *Mammillaria* at genus level. The name was first applied to a genus of algae in 1809 by John Stackhouse. At the International Botanical Congress in 1930, the name *Mammillaria* was conserved for the cactus genus. When Haworth described *Mammillaria*, he renamed Linnaeus' original species *M. mammillaris* to *M. simplex* so as to avoid a tautonym. Although tautonyms are invalid under the International Code for Botanical Nomenclature (Greuter et al., 2000), the name *Mammillaria mammillaris* is not considered to be a tautonym because the species name does not exactly match the genus name, thus Haworth's name *M. simplex* is a later homonym and therefore invalid.

Early Classifications of Mammillaria

In 1837, Pfeiffer proposed an infrageneric classification of *Mammillaria* (Pfeiffer, 1837). Pfeiffer's system of classification (summarized in Figure 3-1) utilized two subgeneric ranks above that of species. The highest subgeneric rank (denoted with "§" in his treatment) divided the genus *Mammillaria* into two groups based upon spine characteristics. The *Homeoacanthae* were characterized by uniform spination in contrast to the *Heteracanthae* which had differentiated spines. Pfeiffer divided the *Homoeacanthae* and the *Heteracanthae* into five subgroups each, a number of which (eg. *Polyedrae* and *Stylothelae*) are familiar names today. However, two of Pfeiffer's subgroup names at this level – *Conothelae* and *Brachythelae* – are included in both the *Homoeacanthae* and *Heteracanthae.*

Salm-Dyck (1845) produced a classification of *Mammillaria* with three ranks between genus and species (Figure 3-1). These ranks were denoted with similar symbols to those that had been used in the classification of Pfeiffer's. Whereas Pfeiffer (1837) only had two upper-level groups within *Mammillaria* (*Homoeacanthae* and *Heteracanthae*), Salm-Dyck's infrageneric classification divided the species of *Mammillaria* among eight major groups: *Longimammae, Crinitae, Heteracanthae, Subsetosae, Centrispinae, Angulares, Stelligerae* and *Aulacothelae.* Of these groups, the *Heteracanthae* encompassed the greatest number of species of *Mammillaria*, and this group along with the *Angulares* and *Aulacothelae* were divided into a number of lower-level groups (denoted in his publication with asterisks). Salm-Dyck further divided the *Mammillaria* species in the *Tetragonae* group of *Angulares* among the *Tetracanthae* and *Hexacanthae* (these groups denoted by double asterisks).

In 1856, George Engelman, a physician based in St Louis, Missouri, published a summary of the cacti of the United States (Engelmann, 1856). Although this publication was not intended to be a thorough treatment of *Mammillaria*, Engelmann explicitly divided and described two subgenera – *Eumamillaria* and *Coryphantha* (see Figure 3-1). The main distinguishing features that Engelmann used to separate his subgenera the age of flower-pro-

Pfeiffer (1837)
§ *Homoeacanthae*
Tenues, ramosae
Conothelae
Brachythelae
Polyedrae
Longimammae
§ *Heteracanthae*
Microthelae
Conothelae
Brachythelae
Stylothelae
Gibbosae

Salm-Dyck (1844)
§ *Longimammae*
§ *Crinitae*
§ *Heteracanthae*
Polyacanthae
Leucocephalae
Chrysacanthae
Discolores
§ *Subsetosae*
§ *Centrispinae*
§ *Angulares*
Tetragonae
Polyedrae
Phymatothelae
Macrothelae
§ *Stelligerae*
§ *Aulacothelae*
Glanduliferae
Eglandulosae

Engelmann (1857)
subg. *Eumamillaria*
§ *Polyacanthae*
§ *Crinitae*
§ *Setosae*
§ *Centrispinae*
§ *Longimammae*
subg. *Coryphantha*
§ *Albiflorae*
§ *Faviflorae*
§ *Rubriflorae*
subg. *Anhalonium*

Schumann (1898)
subg. *Coryphantha*
series *Aulacothelae*
series *Glanduliferae*
subg. *Dolichothele*
series *Longimammae*
subg. *Cochemiea*
series *Exsertae*
subg. *Eumamillaria*
section *Hydrochylus*
series *Leptocladodae*
series *Candidae*
series *Stylothelae*
series *Polyacanthae*
series *Ancistracanthae*
series *Heterochlorae*
section *Galactochylus*
series *Elegantes*
series *Leucocephalae*
series *Macrothelae*
series *Tetragonae*
series *Polyedrae*

Figure 3-1. Early infrageneric circumscriptions of the genus *Mammillaria*. See text for details and explanations. Abbreviations: subg. = subgenus. Symbol § = section.

ducing tubercles and the presence of furrows on the tubercles. In subgenus *Eumammillaria*, Engelmann described the flowers as being produced from tubercles of the previous year, all tubercles remaining completely unfurrowed, whereas in subgenus *Coryphantha*, the flowers develop in tubercles produced during the current year, and tubercles are furrowed or grooved. During the decade following Engelmann's publication, Charles Lemaire came to the opinion that the differences between the two subgenera of *Mammillaria* were of sufficient significance to warrant the elevation of subgenus *Coryphantha* to the rank of genus in its own right (Lemaire, 1868).

Just prior to the turn of the twentieth century, Schumann published what is possibly one of the first detailed monographs of the cactus family. This publication, *Gesamtbeschreibung der Kakteen* (Schumann, 1898), was a thorough treatise of cactus knowledge to date and included an overview of morphology and a detailed classification of the family. Within the genus *Mammillaria* (which included Lemaire's genus *Coryphantha*), Schumann erected an elaborate system of infrageneric taxa consisting of subgenera, sections and series (summarized in Figure 3-1). Whereas previous authors (Pfeiffer, 1837; Salm-Dyck, 1845) had not explicitly stated ranks in their infra-generic classifications of *Mammillaria*, Schumann, who drew-upon the names published by Pfeiffer, Salm-Dyck and Lemaire, was quite explicit in stating the rank of each of his names. For this reason, the names published by Schumann can be treated as the first subgeneric *Mammillaria* names validly published under the International Code of Botanical Nomenclature and have priority over those that were published previously (Hunt, 1971).

Besides the subgenera *Coryphantha* and *Eumamillaria*, Schumann recognized two other subgenera in *Mammillaria*. The subgenus *Dolichothele* K. Schum. included the single series *Longimammae* and two species (*M. sphaerica* Dietr. and *M. longimamma* DC). Schumann placed in subgenus *Cochemiea* (Brandegee) K. Schum. the Baja California species of *Mammillaria* that possess cylindrical, often elongate bodies and produce narrowly tubular, bilabiate flowers. He also included within this subgenus *Mammillaria senilis* Loddiges ex Salm-Dyck,

Table 3-1. Generic circumscriptions of the genus *Mammillaria*. See text for details and explanations. The classifications of Hunt and Lüthy are their classification of non-*Mammillaria* taxa. These taxa are marked as NA in the table.

Schumann (1898)	Britton & Rose (1923)	Berger (1926, 1929)	Buxbaum (1951b, 1958)	Backeberg (1966)	Hunt (1987)	Lüthy (1995, 2001)
Mammillaria	Neomammillaria	Mammillaria	Mammillaria	Mammillaria	Mammillaria	Mammillaria
	Coryphantha	Coryphantha	Coryphantha	Coryphantha	NA	NA
	Escobaria	Coryphantha	Escobaria	Escobaria	NA	NA
	Neobesseya	Coryphantha	Neobesseya	Neobesseya	NA	NA
	Mamillopsis	Mamillopsis	Mamillopsis	Mamillopsis	Mammillaria	Mammillaria
	Cochemiea	Cochemiea	Cochemiea	Cochemiea	Mammillaria	Mammillaria
	Bartschella	Mammillaria	Bartschella	Bartschella	Mammillaria	Mammillaria
	Dolichothele	Mammillaria	Dolichothele	Dolichothele	Mammillaria	Mammillaria
	Phellosperma	Mammillaria	Phellosperma	Phellosperma	Mammillaria	Mammillaria
	Solisia	Solisia	Solisia	Solisia	Mammillaria	Mammillaria
		Porfiria	Porfiria	Porfiria	Mammillaria	Mammillaria

a caespitose species possessing irregular rather than bilabiate flowers from mainland Mexico. The majority of *Mammillaria* species were placed by Schumann into the subgenus *Eumamillaria*. Unlike the subgenera *Dolichothele* and *Cochemiea*, which included a single series each, subgenus *Eumamillaria* was divided into two sections (*Hydrochylus* K. Schum. and *Galactochylus* K. Schum.) based mainly on the presence of watery or milky sap in the plant body. Sections *Hydrochylus* and *Galactochylus* were further split into six and five series respectively. It appears that Schumann may have had a very early phylogenetic insight into the genus *Mammillaria*, for later, in the supplement of his publication (Schumann, 1898), he admits that the genus may be polyphyletic.

Post-Schumann Classifications of Mammillaria

In the first two decades of the twentieth century, the number of species of *Mammillaria* described had increased to such an extent that Britton and Rose (1923) believed the genus was ripe for splitting (see Figure 3-2). In their subtribe *Coryphanthanae*, Britton and Rose included 14 genera, of which all but two [*Ancistrocactus* B. & R., and *Thelocactus* (Schumann) B. & R.] included species once placed in the genus *Mammillaria*. The genus *Mamillopsis* Morren ex B. & R. contained but a single species, *M. senilis* Loddiges ex Salm-Dyck, whose actinomorphic flowers distinguished this species from inclusion in *Cochemiea*. Likewise the scaly floral-tube and other floral differences were considered sufficient to exclude it from species of *Mammillaria*. Unlike Schumann, Britton and Rose accepted the generic status of *Dolichothele* and *Coryphantha*, and actually cleaved two new genera (*Neobesseya* B. & R. and *Escobaria* B. & R.) from the latter. The genus *Bartschella* B. & R. was treated as distinct by Britton and Rose from other species of *Mammillaria* due to the presence of large flowers, black seed and circumscissile fruit. Another new genus erected by Britton and Rose from Schumann's subgenus *Eumamillaria* was *Phellosperma* B. & R. Containing only a single species, *P. tetrancistra* (Engelmann) B. & R. was named for the 'corky' appendage on the seed coat which was not described elsewhere in *Mammillaria*. The final genus created by Britton and Rose was the monotypic

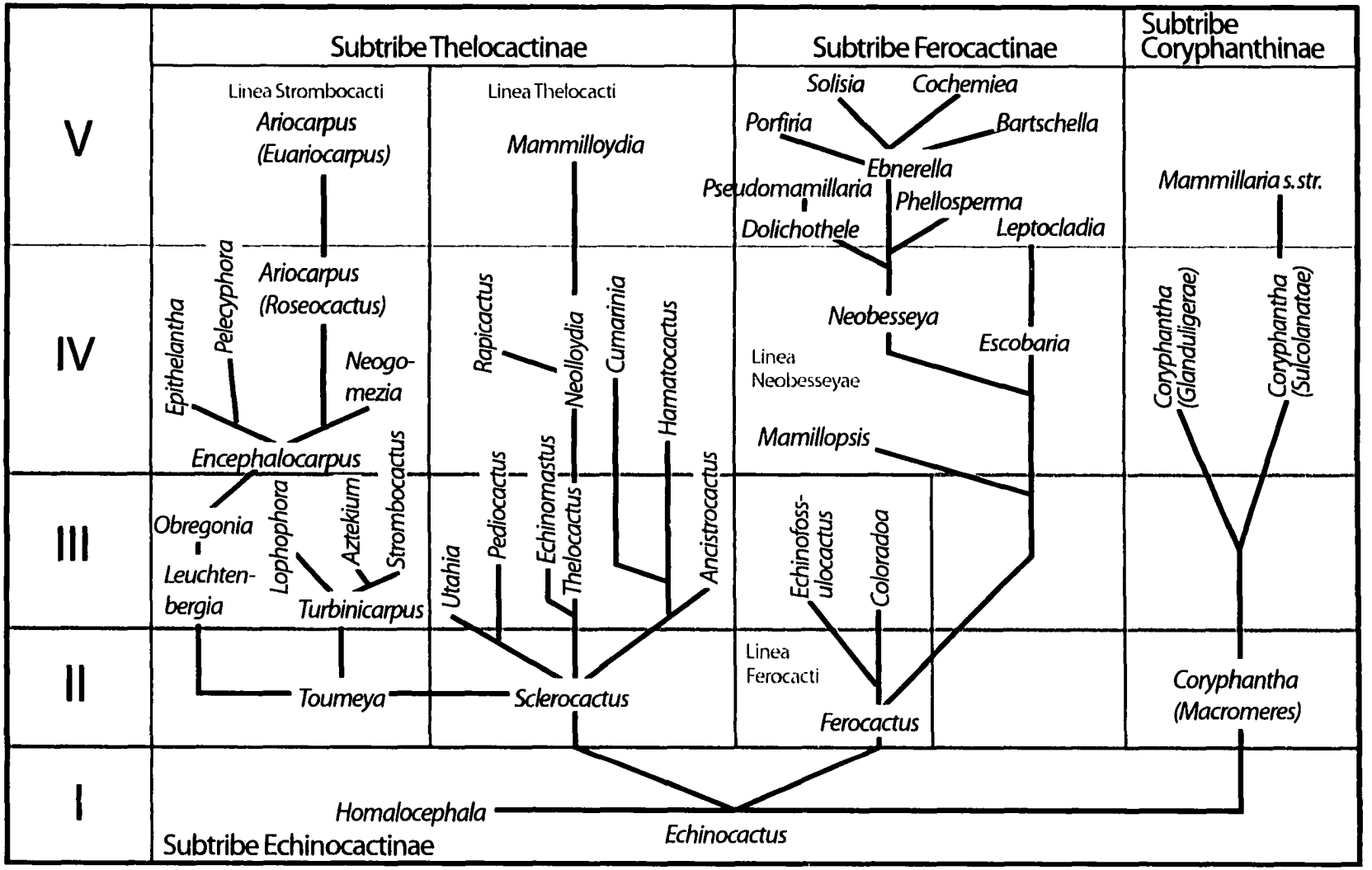

Figure 3-2. Buxbaum's phylogeny of Tribe Echinocacteae (Cacteae). The three main branches form the subtribes Thelocactinae, Ferocactinae and Coryphanthinae, all of which evolved from the 'primordial' stage subtribe Echinocactinae through a number of evolutionary stages. The evolutionary stages in Buxbaum's phylogeny are: I - primordial; II - connecting stage; III - transitory stage; IV - Coryphantha stage; V - *Mammillaria* stage. Figure is redrawn from Buxbaum 1951b and amended according to Buxbaum (1958).

Solisia B. & R., the single species *S. pectinata* (B. Stein) B. & R. having previously been treated as in either *Mammillaria* or *Pelecyphora.*

A final, and important, note about Britton and Rose's work on *Mammillaria* is that they were the first authors to specifically confront the fact that *Mammillaria* Haworth is a later homonym of *Mammillaria* Stackhouse, a genus of algae. For this reason, Britton and Rose renamed the cactus genus *Mammillaria* as *Neomammillaria* B. & R. It was not until the 1930 International Botanical Congress that Haworth's name was conserved and accepted under the International Code of Botanical Nomenclature.

Shortly after Britton and Rose had published their work on the Cactaceae, Berger (1926) published a classification of the family that was based on the Britton and Rose system with a few refinements. For the first time, as part of his classification, Berger attempted to outline his system using dendrograms to show relationships between various taxa. Within his tribe *Mamillariae* Berger, the genera *Bartschella*, *Neomammillaria* and *Dolichothele* are treated as closely related genera, with *Phellosperma* as a more distantly related genus. Berger also treats *Cochemiea* as a distinct genus, but in its own tribe implying that it was derived from ancestral members of tribe *Mamillariae.* Whereas Britton and Rose had chosen not to produce an infrageneric classification of *Mammillaria*, Berger, in his later publication 'Kakteen' (Berger, 1929), subsumed members of his tribe *Mammillariae* (*Dolichothele, Bartschella* and *Phellosperma*) within *Mammillaria*, accepting much of Schumann's earlier infrageneric classification.

As an advocate of Britton and Rose, Craig (1945) chose not to adopt a strict framework of sections and series within *Mammillaria*, instead opting to arrange the species based on a system of comparative keys in what he describes as a "modification of the Britton and Rose System" (Craig, 1945: p. 3). However, Craig opted for a more narrowly circumscribed *Mammillaria* than Britton and Rose, recognizing *Bartschella* and *Dolichothele* as distinct genera, yet not giving generic status to *Phellosperma.*

Franz Buxbaum undertook a detailed study of cactus morphology with the intentions of gaining insight into the phylogenetics of the family, especially his tribe Euechinocactinae (= tribe Cacteae). Like Schumann (1898), Buxbaum clearly felt that *Mammillaria* was polyphyletic. Buxbaum (1951b) suggested that within the North American barrel cacti (which includes *Mammillaria*), there had been an evolutionary trend from larger to smaller plants, through a number of intervening stages of evolution until the final stage – the '*Mammillaria* Stage' was reached (see Figure 3-3). Plants that had attained this stage of evolution were characterized by an appearance similar to that of the genus *Mammillaria*. Buxbaum was quick to point out that these stages occurred in several, quite unrelated groups in the cactus family, and that genera belonging in the same stage of evolution are related only if they belong within the same lineage. For this reason, Buxbaum reasoned that *Mammillaria* in the sense of Schumann (1898) was polyphyletic. Taking the 'micro-genera' approach that had been adopted by Britton and Rose (1923), and the earlier treatment by Berger (1926), Buxbaum (1951a) presented a narrow circumscription of *Mammillaria*, which represented the '*Mammillaria*-stage' representatives of his *Linea Coryphanthanae* (subtribe *Coryphanthinae* Buxbaum 1958). Three new genera were described by Buxbaum (1951a): *Leptocladia* represented species derived from the 'Coryphantha-stage' genus *Escobaria; Pseudomammillaria* which was derived from *Dolichothele*; and *Ebnerella*, which represented '*Mammillaria*-stage' species derived from the genus *Neobesseya* Britton & Rose. The genera *Porfiria, Solisia, Cochemiea* and *Bartschella* were considered by Buxbaum (1951b) to be derived from *Ebnerella.*

Within a few years of his phylogenetic classification of the '*Mammillaria*-stage,' Buxbaum (1954) became aware of a previously published genus name – *Chilita* Orcutt which took priority over the genus name *Ebnerella.* For this reason, Buxbaum (1954) transferred members of *Ebnerella* to the genus *Chilita.* Although he had attempted to correct the polyphyletic nature of *Mammillaria*, using a narrow circumscription of the genus that included only species derived from the '*Coryphantha*-line' rather than his *Linea Neobesseya*, Buxbaum had not actually examined *Mammillaria mammillaris* (L.) Karsten. When he finally man-

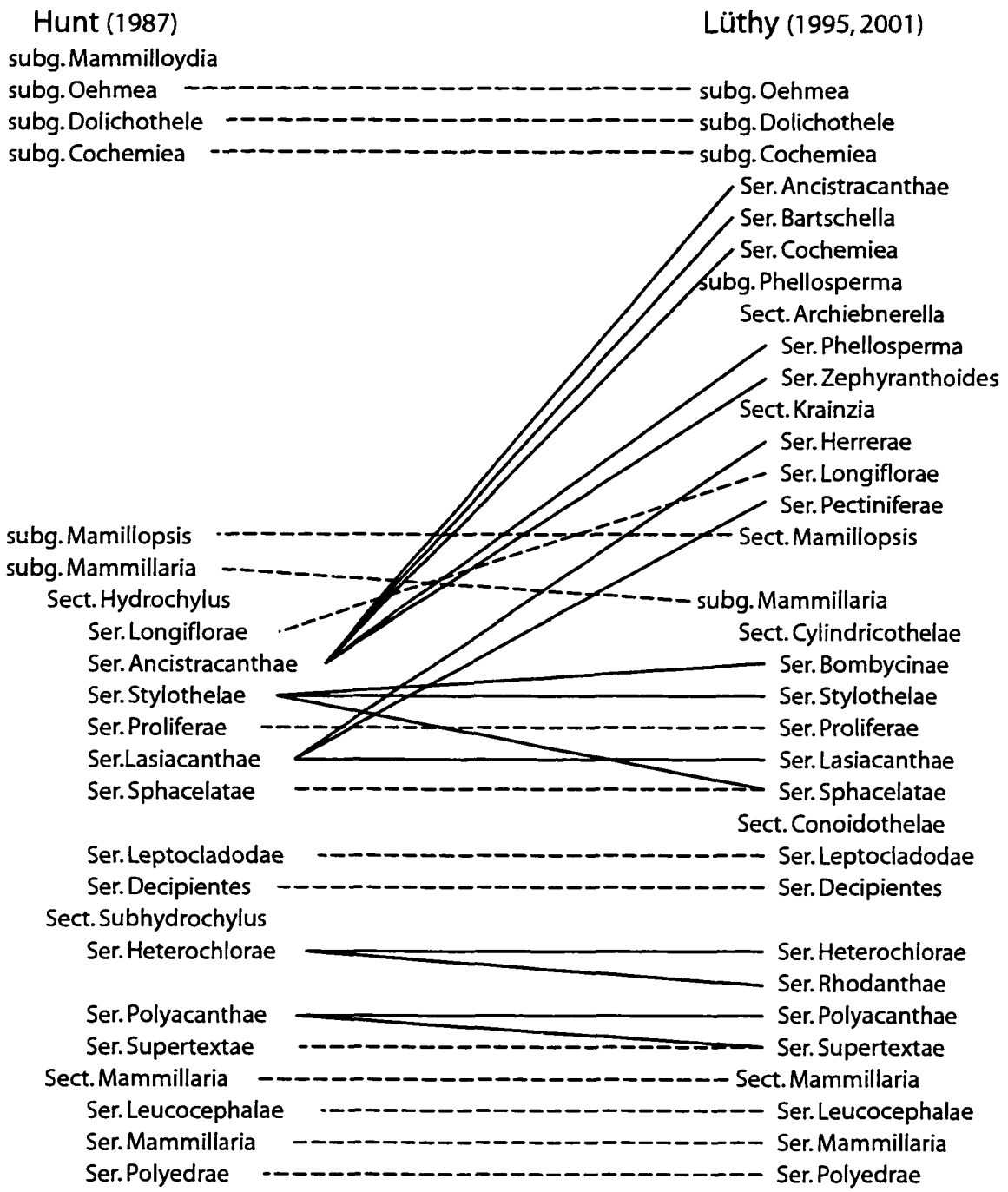

Figure 3-3. Comparison of Hunt's (1987) infrageneric classification of *Mammillaria* with that of Lüthy (1995, 2001). subg. = subgenus; Sect. = section; Ser. = series. The dashed lines indicate infrageneric groupings with similar circumscriptions between the two classifications, solid lines show circumscriptional differences between the two classifications.

aged to do this, he discovered that this species actually belonged as a member of his *Linea Neobesseya* and not *Linea Coryphantha* with which he had grouped all other species of *Mammillaria* (Buxbaum, 1956a, b).

Working during the 1960's and 1970's, David Hunt completely revised the infra-generic classification of *Mammillaria.* Believing that a conservative approach to generic delimitations was more appropriate than numerous segregate genera, Hunt (1971) avoided the classification systems of Britton and Rose (1923), and Buxbaum (1951a; 1954; 1963) and went back to that of Schumann (1898) to form the basis for his classification of *Mammillaria.* However, Hunt did not dismiss the work of Buxbaum completely and acknowledged that the morphological studies of Buxbaum represented a significant contribution to the understanding of the cacti. For this reason, Hunt (1971; 1977a; 1977b; 1977c) attempted to produce an infra-generic classification of *Mammillaria* that included aspects of the classifications of both Schumann and Buxbaum.

As a starting point in his classification, Hunt (1971) accepted Schumann's (1898) subgenera *Dolichothele, Cochemiea,* and *Eumammillaria* (renamed as subgenus *Mammillaria* in accordance with the International Code of Botanical Nomenclature). To these subgenera Hunt added the subgenera *Mamillopsis* Morren and *Mammilloydia* (Buxbaum) Moran. Hunt's rationale behind subsuming the genera *Dolichothele, Cochemiea, Mamillopsis* and *Mammilloydia* into *Mammillaria* was that their recognition was largely based upon seed coat color and morphology. Thus to accept generic boundaries based on such a narrow character-group meant that many other groups within *Mammillaria* sensu stricto would also need to be recognized at genus level.

In 1977, Hunt published a number of papers in which he further modified his infra-generic classification of *Mammillaria* (Hunt, 1977a; 1977b; 1977c). The genus *Oehmea* Buxbaum was recognized as distinct from other *Mammillaria* due to the rugose nature of the seed testa. Hunt had realized that the seed was also pitted and included this genus within subgenus *Dolichothele* (Hunt, 1971). However he later accepted that differences in habit,

tubercles, flowers and fruit warranted recognition of *Oehmea* at subgenus level (Hunt, 1977a). In his final revised classification of *Mammillaria*, Hunt (1981) recognized six subgenera, three sections and 14 series within *Mammillaria*. This classification has been used as the basis for subsequent treatments of the genus by a number of subsequent authors including Hunt (1987), and Pilbeam (1999).

The most recent major contributor to *Mammillaria* taxonomy was the Swiss researcher Jonas M. Lüthy. At a time when taxonomy was becoming strongly cladistic and the use of DNA sequence data was blossoming, Lüthy (1995; 2001) remained steadfast in the belief that detailed morphological studies combined with phenetic analyses could be used to provide information on the taxonomy of *Mammillaria*. To this end, Lüthy utilized an impressive array of characters (ninety in total that included mainly morphological but also chemical and 'ecological' characters for 115 species, subspecies, varieties and forms) which he felt allowed for an informed judgment of character usefulness for classification based on assumed autapomorphies, synapomorphies and plesiomorphies. Unlike the taxonomic revision of *Mammillaria* by Hunt which was based on the Schumann system, Lüthy followed the phenetic approach of excluding all previous conceptions about classification, in essence beginning with 'a clean slate' in terms of an infra-generic classification. The only notable assumptions that Lüthy made regarding *Mammillaria* was its generic circumscription which like Hunt excluded members of *Coryphantha*, but differed in that *Mammillaria candida* was excluded from *Mammillaria* and treated as *Mammilloydia candida*.

Using two-dimensional principal component analyses, Lüthy succeeded in discriminating between 20 major phenons in *Mammillaria* which fell into three major phenon groups – 'primitive', 'intermediate' and 'modern' mammillarias. The infra-generic classification produced by Lüthy from his phenetic analyses divided the genus into four subgenera, seven sections and 22 series. It is interesting to note that Lüthy did not include data from *Mammillaria beneckei* Ehrenb. in his analysis. Buxbaum had treated this taxon as *Oehmea beneckei* and Hunt reassigned this genus into *Mammillaria*, relegating Buxbaum's genus *Oehmea* to

subgeneric level which Lüthy also placed in his classification. Figure 3-4 summarizes the main differences between Hunt's (1987) and Luthy's (1995, 2001) classifications of *Mammillaria*.

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CHAPTER 4

Molecular Systematics of Tribe Cacteae (Cactaceae: Cactoideae): A Phylogeny Based on *rpl*16 Intron Sequence Variation

A paper published in Systematic Botany 27(2): 257-270, 2002.[1]

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ABSTRACT

Parsimony analysis of plastid *rpl*16 sequences from 62 members of Tribe Cacteae, and four outgroup taxa yielded 1296 equally parsimonious trees of length 666. Strict consensus evaluation of these trees established a highly pectinate topology, which delimited clades within the tribe that correspond to several previously considered generic groups. *Aztekium* and *Geohintonia*, which manifest ribs in their stem morphology were shown to represent an early divergence in the tribe, forming a sister group to remaining members of the tribe. Clades containing other genera having ribbed stems also are basal to those that develop tubercles. The most derived clade forms a distinct group of typically small stemmed species with tubercular stem morphology. Within *Mammillaria*, species formerly placed in the genus *Cochemiea* and members of the Series *Ancistracanthae* formed a well-supported, sister clade to the remaining members of *Mammillaria*. Length variation of the intron in two members of *Mammillaria* series *Stylothelae* was also observed.

Communicating Editor: Thomas G. Lammers

INTRODUCTION

Buxbaum (1958b) first described the tribe Cacteae (as the Echinocacteae) as a 'clear-cut phylogenetic unit' in which he included all of the short-columnar or globose cacti with spineless flowers native to North America, with the notable exception of the genus *Astrophytum* Lemaire, which he considered part of the Notocacteae. The tribe is in considerable taxonomic flux, and poor generic delineation means that the exact number of genera is uncertain. Twenty-three genera are recognized in the CITES Cactaceae checklist (Hunt 1999), although at least 34 other genera have been described. Hunt (1999) accepts 314 species (plus 224 provisionally accepted species) in the tribe, whereas Anderson (2001) recognizes 26 genera and

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[5] Major Professor.

384 species. The geographic range of the Cacteae extends from western Canada (*Escobaria vivipara* (Nuttall) Buxbaum) to Colombia, Venezuela, and the Caribbean (*Mammillaria colombiana* Salm-Dyck and *M. mammillaris* (L.) Karsten), with maximal diversity in Mexico.

Characterized as globular or depressed to short columnar cacti, members of the Cacteae range in size from dwarf (*Turbinicarpus* (Backeberg) Backeberg and Buxbaum and some *Mammillaria* Haworth) to gigantic (*Ferocactus* Britton and Rose and *Echinocactus* Link and Otto). Stems may be either ribbed, as in *Echinocactus*, or tuberculate as in *Coryphantha* (Engelmann) Lemaire. Zimmerman (1985) states that ribs and tubercles are mutually exclusive terms, although a number of intermediates are found. He recommends the use of the term podarium, suggesting that in reality ribs are series of podaria joined end-to-end. Tubercles, however, represent free or distinct podaria. This terminology allows for intermediacy between ribs and tubercles. The size and shape of tubercles range from long and leaf-like (as in *Leuchtenbergia* Hooker, *Obregonia* Fric, and some species of *Ariocarpus* Scheidweiler) to broad with shallow axils, as in *Turbinicarpus*.

Areoles may be borne on the ribs, or in the case of the tuberculate members, may occur at or near the tubercle apex, or form a groove on the upper surface as in *Coryphantha* and some species of *Escobaria* Britton and Rose. In a number of genera the tubercles are differentiated into spine-bearing areoles at the tubercle apex and floriferous or vegetative areoles in the axils of the tubercles. Buxbaum (1958b) suggested that species with differentiated tubercles such as *Mammillaria* are derived within the tribe. Actinomorphic or, rarely, zygomorphic (*Mammillaria* subgenus *Cochemiea* Brandegee) diurnal flowers arise from the areoles. The pericarpel (in cacti defined as ovary wall plus stem tissue external to the ovary wall) ranges from scaly and woolly to petaloid. Fruits in the Cacteae are fleshy to juicy berries, and the seeds are borne on short, simple funiculi. Since the 1920's, a number of researchers have revised the Cacteae, variously interpreting its classification based on differing concepts of broadly-defined or narrowly circumscribed genera. Table 4-1 lists genera accepted in a number of key treatments of the Cacteae.

Table 4-1. Comparisons of previous treatments for members of the Cacteae.

This paper	Anderson (2001)	Hunt (1999)	Barthlott & Hunt (1993)	Backeberg (1970)	Buxbaum (1958b)	Britton & Rose (1919-1923)
Acharagma	*Escobaria*	*Escobaria*	*Escobaria*	*Escobaria, Gymnocactus*	*Escobaria*	*Not Described*
Ariocarpus	*Ariocarpus*	*Ariocarpus*	*Ariocarpus*	*Ariocarpus, Neogomesia, Roseocactus*	*Ariocarpus*	*Ariocarpus*
Astrophytum	*Astrophytum*	*Astrophytum*	*Astrophytum*	*Astrophytum*	*Astrophytum*	*Astrophytum*
Aztekium	*Aztekium*	*Aztekium*	*Aztekium*	*Aztekium*	*Aztekium*	Not Described
Coryphantha	*Coryphantha*	*Coryphantha*	*Coryphantha*	*Coryphantha, Cumarinia, Lepidocoryphantha*	*Coryphantha, Cumarinia*	*Coryphantha*
Echinocactus	*Echinocactus*	*Echinocactus*	*Echinocactus*	*Echinocactus*	*Echinocactus*	*Echinocactus*
Encephalocarpus	*Pelecyphora*	*Pelecyphora*	*Pelecyphora*	*Encephalocarpus*	*Encephalocarpus*	*Pelecyphora*
Epithelantha	*Epithelantha*	*Epithelantha*	*Epithelantha*	*Epithelantha*	*Epithelantha*	*Epithelantha*
Escobaria	*Escobaria*	*Escobaria*	*Escobaria*	*Escobaria, Neobesseya*	*Escobaria, Neobesseya*	*Escobaria*
Ferocactus	*Ferocactus*	*Ferocactus*	*Ferocactus*	*Ferocactus*	*Ferocactus*	*Ferocactus*
Geohintonia	*Geohintonia*	*Geohintonia*	*Geohintonia*	Not Described	Not Described	Not Described
Glandulicactus	*Sclerocactus*	*Sclerocactus*	*Sclerocactus*	*Glandulicactus*	*Hamatocactus*	*Hamatocactus*
Homalocephala	*Echinocactus*	*Echinocactus*	*Echinocactus*	*Homalocephala*	*Homalocephala*	*Homalocephala*
Leuchtenbergia	*Leuchtenbergia*	*Leuchtenbergia*	*Leuchtenbergia*	*Leuchtenbergia*	*Leuchtenbergia*	*Leuchtenbergia*
Lophophora	*Lophophora*	*Lophophora*	*Lophophora*	*Lophophora*	*Lophophora*	*Lophophora*
Mammillaria	*Cochemiea, Mammillaria, Mammilloydia*	*Mammillaria, Mammilloydia*	*Mammillaria, Mammilloydia*	*Bartschella, Cochemiea, Dolicothele, Krainzia, Mamillopsis, Mammillaria, Phellosperma, Solisia,*	*Cochemiea, Dolichothele, Leptocladodia, Mamillopsis, Mammillaria, Mammilloydia, Oehmea, Pseudomamillaria*	*Bartschella, Cochemiea, Dolichothele, Mamillopsis, Neomammillaria, Phellosperma, Solisia*
Neolloydia	*Neolloydia*	*Neolloydia*	*Neolloydia*	*Neolloydia*	*Neolloydia*	*Neolloydia*
Obregonia	*Obregonia*	*Obregonia*	*Obregonia*	*Obregonia*	*Obregonia*	Not Described
Ortegocactus	*Ortegocactus*	*Ortegocactus*	*Ortegocactus*	*Ortegocactus*	Not Described	Not Described
Pediocactus	*Pediocactus*	*Pediocactus*	*Pediocactus*	*Navajoa, Pediocactus, Pilocanthus, Utahia*	*Pediocactus, Utahia*	*Pediocactus, Utahia*
Pelecyphora	*Pelecyphora*	*Pelecyphora*	*Pelecyphora*	*Pelecyphora*	*Pelecyphora*	*Pelecyphora*

Table 4-1. Continued.

This paper	Anderson (2001)	Hunt (1999)	Barthlott & Hunt (1993)	Backeberg (1970)	Buxbaum (1958b)	Britton & Rose (1919-1923)
Sclerocactus	*Sclerocactus*	*Sclerocactus*	*Sclerocactus*	*Coloradoa, Echinomastus, Gymnocactus, Sclerocactus, Toumeya*	*Ancistrocactus, Coloradoa, Echinomastus, Sclerocactus*	*Ancistrocactus, Sclerocactus, Toumeya*
Stenocactus	*Stenocactus*	*Stenocactus*	*Stenocactus*	*Echinofossulocactus*	*Echinofossulocactus*	*Echinofossulocactu*
Strombocactus	*Strombocactus*	*Strombocactus*	*Strombocactus*	*Strombocactus*	*Strombocactus*	*Strombocactus*
Thelocactus	*Thelocactus*	*Thelocactus*	*Thelocactus*	*Echinomastus, Thelocactus*	*Thelocactus*	*Echinomastus, Thelocactus*
Turbinicarpus	*Turbinicarpus*	*Turbinicarpus*	*Neolloydia*	*Gymnocactus, Turbinicarpus*	*Rapicactus, Toumeya*	*Neolloydia*

Britton and Rose (1919–1923) did not recognize the Cacteae as a discrete entity. Within their tribe Cereeae (equals subfamily Cactoideae), they divided the barrel cacti into two subtribes, Echinocactinae and Coryphanthinae. Their subtribe Echinocactinae included all ribbed barrel cacti from both North and South America, which manifest a generally low growing, globular habit. The North American barrel cacti that share the character of possessing tubercles were placed into the subtribe Coryphanthinae, although some taxa with true tubercles or modified tubercles, such as *Pediocactus* Britton and Rose, *Ariocarpus*, and *Lophophora* Coulter were placed within the ribbed subtribe Echinocactinae. It is evident that Britton and Rose (1919–1923) realized that mutually exclusive suites of morphological characters could not be used to delineate subtribes within their tribe Cereeae, accepting that boundaries between subtribes Echinocereanae, Echinocactinae and Coryphanthinae were not clearly defined.

Using an underlying principle of determining taxonomic groups based on inferred phylogenetic relatedness, Buxbaum (1958b) described the North American barrel cacti (with minor exceptions) at the rank of tribe (Cacteae), and defined this group by bringing together Schumann's earlier tribe Echinocacteae (Schumann 1899) and Britton and Rose's subtribes

Echinocactanae and Coryphanthanae. With the exception of *Astrophytum* (which he placed into the tribe Notocacteae), Buxbaum (1958b) recognized 36 genera in the tribe and regarded this group of North American barrel cacti as a distinct phylogenetic unit. Within his tribe Cacteae, four subtribes were defined based upon seed morphology: 1) the Echinocactinae, with a smooth, hard, black testa with conspicuous perisperm; 2) the Thelocactinae, with a verrucose, mostly black testa becoming secondarily smooth or 'spotted'; 3) the Ferocactinae, with a pitted or reticulate testa; and 4) the Coryphanthinae, with a smooth, brown testa.

In contrast to Buxbaum's phylogenetically-based classification, Backeberg's (1970) classification of the cacti used a complex system of infrafamilial ranks including semitribes, subtribes, groups, and subgroups. This classification was never intended to be phylogenetic. Britton and Rose's tribe Cereeae was split, largely based on geographic origins of the plants, into the North and South American semitribes Boreocereeae and Austrocereeae, respectively. Ignoring Buxbaum's (1958b) tribe Cacteae, Backeberg created the subtribe Boreocactinae to accommodate the North American barrel cacti, which was further divided into two groups based on flower position: 1. The Boreoechinocacti has flowers borne from undifferentiated (vegetative vs. flowering) areoles (the Boreoechinocacti were still further divided into two subgroups, the Euboreoechinocacti and the Mediocoryphanthae) and 2. The Mammillariae, which has differentiated areoles (e.g. flowers borne in tubercle axils), with three subgroups, Coryphanthiae, Mediomammillariae, and Eumammillariae. In total, Backeberg's subtribe Boreocactinae included 48 genera, consistent with his philosophy of recognizing many genera with few species in each. In modern taxonomic treatments, many of these "micro-genera" have been united into more broadly defined groups; for example *Ariocarpus* was expanded by Anderson (1960, 1962) to include *Roseocactus* Berger and *Neogomesia* Castañeda, and the genera *Porfiria* Bödecker, *Krainzia* Backeberg, *Phellosperma* Britton and Rose, *Dolichothele* (Schumann) Britton and Rose, *Bartschella* Britton and Rose, *Mamillopsis* Morren ex Britton and Rose, and *Cochemiea* (Brandegee) Walton were subsumed into the genus *Mammillaria* by Hunt (1971, 1977a, b; 1981).

Besides the treatment by Buxbaum (1958b), there has been only one other attempt at a phylogenetic evaluation of the tribe Cacteae. In his unpublished Ph.D. thesis, Zimmerman (1985) presented a cladistic study of the tribe based on an analysis of morphological characters, the majority of which are derived from the study of floral structures. Zimmerman suggested that the Pachycereeae and the Notocacteae probably represent the closest outgroups to tribe Cacteae, and that the tribe likely had its origins in South America, sharing a sister-group relationship with the Notocacteae. Influenced by Buxbaum's (1958b) treatment, both Barthlott (1977) and Zimmerman (1985) questioned the placement of *Astrophytum* in the Cacteae, noting significant differences in seed morphology. Zimmerman concluded that, with the possible exception of this genus, the Cacteae formed a monophyletic unit. Furthermore, Zimmerman (1985) placed *Astrophytum* within a clade together with *Echinocactus* and *Homalocephala* Britton and Rose that shows a sister-group relationship to other members of the tribe. Despite problems associated with morphological plasticity in the tribe, Zimmerman made a number of insightful conclusions, for example that *Escobaria*, *Ortegocactus*, *Mammillaria*, and *Coryphantha* sensu stricto are derived from a *Mammillaria*-like rather than a *Ferocactus*-like ancestor.

In their treatment of the genera of the Cactaceae, Barthlott and Hunt (1993) united a number of Cacteae genera, recognizing 22 genera in total. *Homalocephala* was included within *Echinocactus*, and the genera *Oehmea* Buxbaum, *Cochemiea*, *Dolichothele*, and *Mamillopsis* were subsumed within *Mammillaria*. Hunt (pers. comm.) doubts that the Cacteae are monophyletic, reasoning that because the globular growth form has arisen independently in several cactus lineages in South America, it has likely also arisen in North American lineages independently.

The primary goals of this investigation were to test monophyly of the tribe, resolve intergeneric relationships in the Cacteae, and to assess monophyly in previously proposed Cacteae genera using chloroplast *rpl*16 intron sequence data. Further, we wished to ascertain relevant outgroup taxa for an ongoing study of the genus *Mammillaria*.

Table 4-2. Species sampled for *rpl*16 study. CANTE = CANTE Botanic Garden, Mexico; UCONN = University of Connecticut; DES = Desert Botanic Garden, Arizona; ISC = Ada Hayden Herbarium, Iowa State University; HNT = Huntington Botanic Garden, California; HUMO = Universidad Autónoma del Estado de Morelos, Mexico; and UNAM = Universidad Autónoma de Mexico, Mexico City.

Taxon	Source/Voucher	GenBank No.
Tribe Cacteae		
Acharagma aguirreana (Glass & Foster) Glass	Mesa Garden—ISC	AF267915
Acharagma roseana (Boed.) Glass	DES 1990-0791-0201—ISC	AF267916
Ariocarpus agavoides (Castañeda) Anderson	C. Glass 6889—CANTE	AF267918
Ariocarpus retusus Scheidw.	C. Glass 6923—CANTE	AF267919
Astrophytum capricorne (Dietrich) Br. & R.	HNT 69033—HNT	AF267920
Astrophytum myriostigma Lem.	HNT 69032—HNT	AF267921
Aztekium hintoni Glass & Fitz Maurice	C. Glass 6647—CANTE	AF267922
Aztekium ritteri (Boed.) Boed.	C. Staples s.n.—ISC	AF267923
Coryphantha pallida Br. & R.	H. Cota 8050—HUMO	AF267926
Echinocactus grusonii Hildm.	R. Wallace s.n.—UCONN	AF267927
Echinocactus horizonthalonius Lem.	M. Mendes 186—CANTE	AF267928
Echinocactus ingens Zucc.	HNT 59498—HNT	AF267929
Encephalocarpus strobiliformis (Werderm.) Berger	HNT 60211—ISC	AF267930
Epithelantha bokei L. D. Benson	DES 1993-0717-0101—ISC	AF267931
Escobaria zilziana (Boed.) Backeb.	DES 1989-0137-0102—DES	AF267932
Ferocactus cylindraceus (Engelm.) Orcutt	Ecker 110—ISC	AF267933
Ferocactus flavovirens (Scheidw.) Br. & R.	H. Cota 8051—HUMO	AF267934
Ferocactus glaucescens (DC.) Br. & R.	HNT 10339—ISC	AF267979
Ferocactus histrix (DC.) Lindsay	H. Cota 8037—CANTE	AF267935
Ferocactus latispinus (Haw.) Br. & R.	H. Cota 8039—CANTE	AF267936
Ferocactus robustus (Link & Otto) Br. & R.	H. Cota 8045—HUMO	AF267974
Ferocactus wislizenii (Engelm.) Br. & R.	L Slauson 112—DES	AF267937
Geohintonia mexicana Glass & Fitz Maurice	C. Glass 6648—CANTE	AF267938
Glandulicactus crassihamatus (Weber) Backeb.	C. Glass 5201—CANTE	AF267939
Glandulicactus uncinatus (Galeotti) Backeb.	C. Glass 6846—CANTE	AF267917
Homalocephala texensis (Hoppfer) Br. & R.	HNT 67080—ISC	AF267940
Leuchtenbergia principis Hook.	HNT s.n.—ISC	AF267941
Lophophora diffusa (Croizat) Bravo	Mesa Garden—ISC	AF267942
Lophophora williamsii (Lem.) J. M. Coult.	D. Martinez s.n.—HUMO	AF267943
Mammillaria beneckei Ehrenb.	DES 1993-0550-0101—DES	AF267944
Mammillaria candida Schweidw.	DES 1957-5907-0101—ISC	AF267945
Mammillaria decipiens Schweidw.	HNT 68830—ISC	AF267946
Mammillaria glassii Foster	HNT 60162—ISC	AF267952
Mammillaria haageana Pfeiffer	H. Cota 8053—HUMO	AF267953
Mammillaria halei Brandegee	HNT 72646—ISC	AF267947
Mammillaria jaliscana (Br. & R.) Boed.	Lau 1050—ISC	AF267948

TABLE 2 (continued).

Taxon	Source/Voucher	GenBank No.
Mammillaria longimamma DC.	DES 1992-0049-0203—DES	AF267950
Mammillaria magnifica Buchenau	HNT—ISC	AF267951
Mammillaria plumosa Weber	HNT 28166—ISC	AF267954
Mammillaria poselgeri Hildm.	DES 1983-0746-1018—ISC	AF267955
Mammillaria senilis Salm-Dyck	Mesa Garden—ISC	AF267956
Mammillaria voburnensis Scheer	Lippold s.n.—UCONN	AF267957
Mammillaria yaquensis Craig	HNT 7715—ISC	AF267958
Neolloydia conoidea (DC.) Br. & R.	C.P. 2—ISC	AF267959
Obregonia dengrii Fric	R. Wallace s.n.—ISC	AF267960
Ortegocactus macdougalii Alexander	R. Wallace s.n.—ISC	AF267961
Pediocactus simpsonii (Engelm.) Br. & R.	C. Butterworth 60—ISC	AF267962
Pelecyphora aselliformis Ehrenb.	DES 1961-6848-0101—DES	AF267963
Sclerocactus brevihamatus (Engelm.) D. R. Hunt	DES 1989-0315-0101—DES	AF267964
Sclerocactus spinosior (Engelm.) Woodruff & L. Benson	Hughes 2—ISC	AF267965
Sclerocactus whipplei (Englem. & Bigelow) Br. & R.	DES 1993-0925-0103—DES	AF267966
Stenocactus crispatus Berger	HNT 46450—HNT	AF267980
Stenocactus lloydii Berger	ex Hort. UCONN—UCONN	AF267977
Stenocactus vaupelianus (Werdem.) F. M. Knuth	DES 1948-1289-0101—DES	AF267978
Strombocactus disciformis (DC.) Br. & R.	H. Sánchez-Mejorada 3603—UNAM	AF267967
Thelocactus conothelos (Reg. & Klein) F. M. Knuth	C-11 AUR	AF267968
Thelocactus hastifer (Werderm. & Boed.) F. M. Knuth	Peter Sharp s.n.	AF267973
Thelocactus macdowellii (Rebut ex Quehl) C. Glass	HNT s.n.—ISC	AF267969
Turbinicarpus gielsdorfianus (Werdermann) John & Riha	HNT 50008—ISC	AF267970
Turbinicarpus pseudomacrochele (Backeb.) F. Buxb. & Backeb.	Brach's Nursery—ISC	AF267971
Turbinicarpus schmiedickianus var. *schwartzii* (Shurly) Glass & Foster	Ex Martiny s.n.—ISC	AF267972
Tribe Browningieae		
Calymmanthium substerile Ritter	HNT 46555—HNT	AF267924
Tribe Notocacteae		
Corryocactus brachypetalus (Vaupel) Br. & R.	HNT 18015—HNT	AF267925
Parodia haselbergii (Haage ex Rümpler) Brandt	ex Hort. —UCONN	AF267975
Tribe Pachycereeae		
Bergerocactus emoryi (Engelm.) Br. & R.	HNT 16514—HNT	AF267976

Materials and Methods

Taxonomic Sampling

A total of 66 taxa were sampled (Table 4-2), including 62 representative taxa from the tribe Cacteae. Two species from tribe Notocacteae and one species from tribe Pachycereeae were also included with members of tribe Cacteae as the ingroup. *Calymmanthium substerile* (tribe Browningieae) was used as the outgroup based upon its basal position within the subfamily Cactoideae (Wallace 2001). Additional phylogenetic analyses of chloroplast DNA variation (Butterworth and Wallace, unpublished) were conducted in which representative taxa were examined from throughout the subfamily, and demonstrated that tribe Cacteae was well supported as a monophyletic group. Specimens were obtained from a number of sources and maintained in the greenhouse prior to DNA extraction. Institutions in which voucher specimens are deposited are also listed in Table 4-2.

DNA Extraction and Purification

Total genomic DNA of representative Cacteae samples was isolated using one of two methods:

1. Modified organelle pellet method suitable for mucilaginous material. Genomic DNA samples were prepared using previously published methods (Wallace 1995; Wallace and Cota 1996) for extraction of nucleic acids from highly mucilaginous plants, briefly summarized as follows: fresh, chlorenchymatous stem tissue was homogenized in 0.35M sorbitol buffer, filtered through Miracloth™ (Calbiochem). The organelles were pelleted, supernatant removed, and pellets were then suspended in 2x CTAB (Doyle and Doyle 1987) for 1 h at 60°C. After partitioning against CHCl3:octanol, 24:1. DNA was isopropanol-precipitated and resuspended for further purification using isopycnic ultracentrifugation in cesium chloride/ ethidium bromide gradients, followed by dialysis against TE.

2. Nucleon Phytopure™ plant and fungal DNA extraction kit for 1g samples (Amersham Life Science). DNA was extracted from living stem tissue according to the manufacturer's recommendations and stored at -20°C in TE buffer.

Amplification and Sequencing

Polymerase chain reaction (PCR) amplification of the *rpl*16 intron was conducted in 100µl reactions using GeneAmp™ PCR Core Reagents (Perkin Elmer), and the amplification primers RP71F and RP1661R (Applequist and Wallace 2000). Each reaction included 20ng of each primer and 5µl of unquantified DNA template. The PCR reactions were conducted in a MJ Research PTC-100 thermal cycler using the following temperature cycling parameters: 1) initial melting at 95°C for 5 min; 2) 24 cycles of the following protocol: 95°C melt for 2 min, 50°C annealing for 1 min, ramp temperature increase of 15°C at 0.125°C per sec, 65°C extension for 4 min; and a final extension step at 65°C for 10 min.

Agarose electrophoresis in TAE was used to confirm the presence of 1.1kb to 1.3kb PCR amplification products. The amplicons were cleaned and concentrated in Microcon 100 spin microconcentrators (Amicon Inc.) following the manufacturer's directions. The products were then quantified in an ultraviolet spectrophotometer and diluted to 50µg/ml for use in sequencing reactions.

Sequence data were obtained using the sequencing primers RP1516R and RP637R (Applequist and Wallace 2000) at concentrations of 5pmol in chain-termination reactions using the ABI Prism Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). We found that dilutions of 1:4 of terminator ready reaction solution gave acceptable reads.

Electrophoresis and automated sequence reading were conducted using Perkin Elmer/ Applied Biosystems automatic sequencing units (ABI Prism 377) at the Iowa State University Nucleic Acid DNA Facility. Sequences typically were 650 or more nucleotides in length. In

a small number of taxa, a poly-T region approximately 400bp from the RP1516R priming site caused extremely poor reads upstream of the RP637R priming site. To overcome this problem, a new primer, RP543F, (5'-TCAAGAAGCGATGGGAACGATGG-3') was designed to run forward from just downstream of the RP637R priming site, overlapping the unreadable section of sequence. Due to extensive poly-A and poly-T regions in Domain I at the 5' end, 150–200bp of the intron sequence could not be obtained using the automated method. Kelchner and Clark (1997) demonstrated low levels of sequence divergence in this region and because it is of limited phylogenetic usefulness, further attempts at obtaining a full length intron sequence were discontinued.

Phylogenetic Analysis

Sequence alignment was carried out using AutoAssembler (Applied Biosystems 1995) and Se-Al (Rambaut 1995). Following an initial Clustal W alignment, sequences were further aligned manually (e.g., Golenberg *et al.* 1993). Insertions/deletions considered to be phylogenetically informative were coded in binary (presence/absence) and added to the end of the data matrix. There were two regions (totalling 61 nucleotides) where alignments were of doubtful homology. These regions were excluded from the analyses. All analyses were carried out using PAUP* 4.0b2 (Swofford 1999). To test the *rpl*16 intron dataset for phylogenetic signal the g-statistic for 10,000 random trees was calculated. According to Hillis and Huelsenbeck (1992) the distribution of lengths of random trees for all topologies provides a 'sensitive' measure of phylogenetic signal within the dataset. Matrices that contain a strong phylogenetic signal show distributions that approach a left-skewed gamma distribution as opposed to a more normal distribution for matrices containing random noise.

Parsimony analyses were done using the heuristic search option. All substitutions and indels were equally weighted. An initial heuristic search using TBR branch swapping saving multiple parsimonious trees (MULTREES ON) was conducted. Random addition searches of

1,000 replicates, saving 100 most parsimonious trees at each step, were undertaken to search for islands of shorter trees. Estimates of decay (Bremer 1988) were obtained using converse constraint trees as implemented using Autodecay (Eriksson and Wikström 1995). Bootstrap values were estimated using the 'fast bootstrap' method for 1,000 replicates. A neighbor-joining analysis was also undertaken using the F81 substitution model.

Results

Sequence length ranged from 650bp in *Mammillaria glassii* and 673bp in *M. magnifica* and *M. haageana* to 935bp in *Ferocactus glaucescens*. Aligned sequence length for the *rpl*16 dataset was 1057bp. The full dataset (including binary-coded indels) totaled 1069 characters. After exclusion of indels and the two regions of doubtful homology, the dataset was 953 characters long, of which 177 were parsimony-informative. The g-statistic for the *rpl*16 dataset is 0.506. This value falls well within the 99% confidence interval (C.I.) for datasets of over 25 taxa and 500 characters (Hillis and Huelsenbeck 1992) and therefore indicates significant phylogenetic structure within the dataset. The data matrix of aligned *rpl*16 sequences is available from the authors.

A heuristic search using PAUP* found 1296 most parsimonious trees with length of 666 steps. There appears to be considerable homoplasy in the *rpl*16 dataset with a C.I. of 0.632 (0.494 excluding uninformative characters). However, a low C.I. may be expected, due in part to the nature of large datasets, thus the retention index (R.I.) gives a more suitable indication of support. In the case of the *rpl*16 dataset, the R.I. (excluding uniformative characters) is 0.699. A random addition search of 100 replicates did not find any islands of shorter trees. The strict consensus tree (Figure 4-1) supports monophyly of the Cacteae, with a decay value of 9 and a bootstrap value of 100%.

Within the Cacteae, the general tree topology resolves a number of clades nested pectinately within each other: 1. "*Aztekium* Clade" consisting of *Aztekium* Bödecker and

Geohintonia Glass and Fitz Maurice (bootstrap 100%, decay 7); 2. "*Echinocactus* Clade"—*Astrophytum*, *Echinocactus*, and *Homalocephala* (bootstrap 62%, decay 2); 3. *Sclerocactus* Britton and Rose (bootstrap 89%, decay 4); 4. "Lophophora Clade"—*Acharagma* (Taylor) Glass, *Lophophora*, and *Obregonia* (bootstrap 87%, decay 5); 5. *Strombocactus disciformis* forms a single lineage; 6. "ATEP Clade"—A weakly supported clade (bootstrap <50%, decay 1) unites *Ariocarpus*, *Turbinicarpus*, *Epithelantha*, and *Pediocactus*; 7. "*Ferocactus* Clade"—consisting of *Ferocactus*, *Ancistrocactus* Britton and Rose, *Leuchtenbergia*, *Echinocactus grusonii*, *Thelocactus* (Schumann) Britton and Rose, and *Glandulicactus* Backeberg (this clade is poorly resolved and poorly supported with bootstrap <50% and decay 1); 8. *Stenocactus* (Schumann) Hill (bootstrap 100%, decay 4); 9. "Mammilloid Clade" including *Pelecyphora* Ehrenberg, *Encephalocarpus* Berger, *Escobaria*, *Coryphantha*, *Neolloydia* Britton and Rose, *Ortegocactus*, and *Mammillaria* (this terminal clade is well-supported with bootstrap 60%, and decay 3).

Analysis of the *rpl*16 data using a neighbor-joining algorithm with the F81 substitution model resulted in an initial tree that was topologically quite congruent with the maximum parsimony tree. There were, however a number of exceptions. *Mammillaria glassii* forms a sister-group to all of the remaining members of the Cacteae in the neighbor-joining tree. This incongruence is caused by differences in sequence length of *Mammillaria glassii* of only 650bp due to a large deletion spanning the region with most informative characters. Other topological differences between the maximum-parsimony and neighbor-joining trees were observed in the placement of members of the *Lophophora* and *Echinocactus* clades of the maximum parsimony tree, which form a single clade in the neighbor-joining tree.

Discussion

Phylogenetic Relationships in the Cacteae

Monophyly of the Cacteae. The phylogeny presented in this paper supports a monophyletic origin for members of the Cacteae as currently circumscribed; no direct relationship

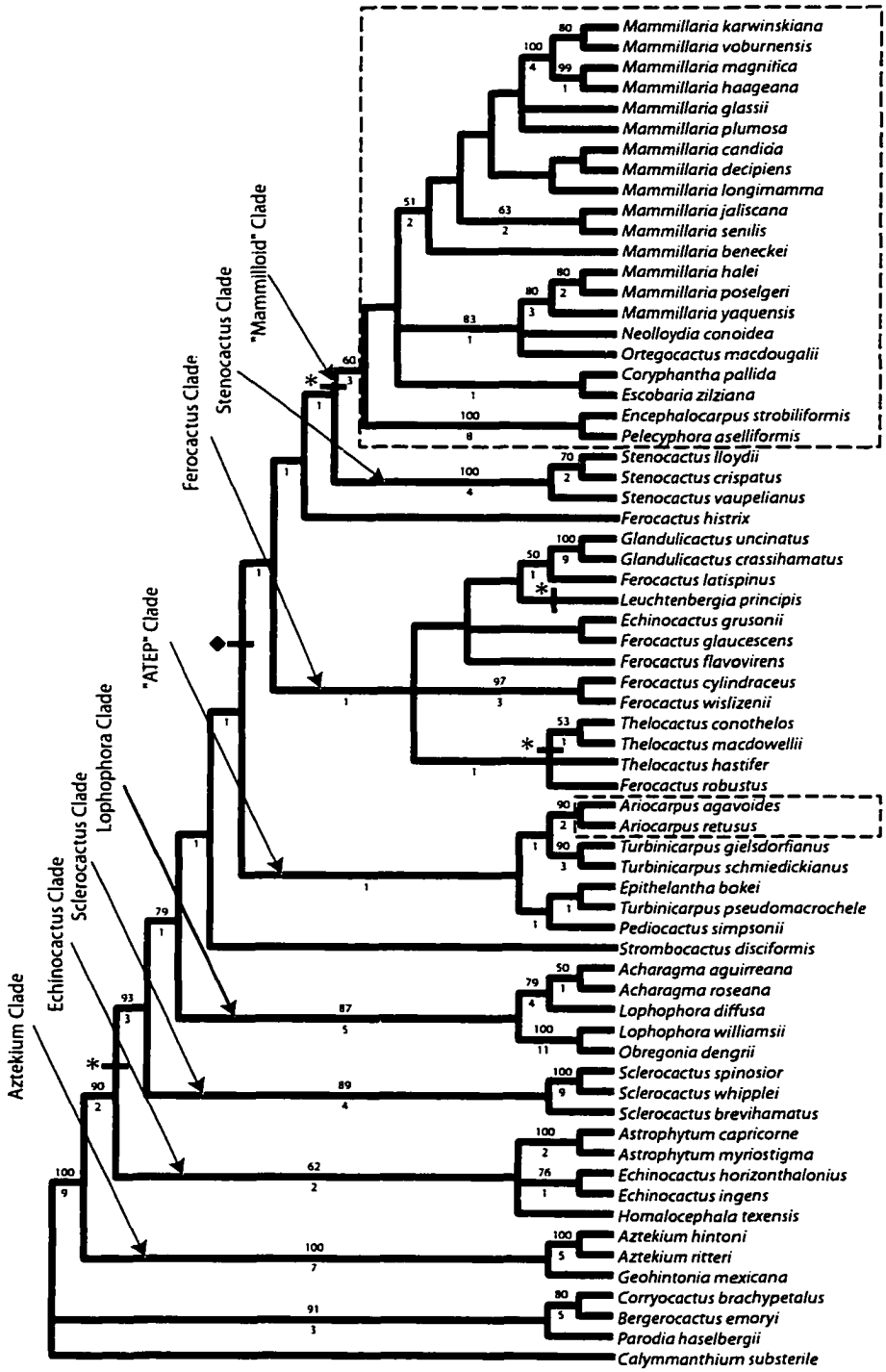

Figure 4-1. Strict consensus of 1296 most parsimonious trees for rpl16 intron sequences. Length = 666 steps, C.I. = 0.632, C.I. (excluding uninformative characters) = 0.494, R.I. (excluding uninformative characters) = 0.699. Bootstrap values over 50% for 1000 replicates are given above the branches. Decay values are shown below the branches. * = switch from ribbed to tubercular stems. ♦ = switch from tubercular to ribbed stems. Boxes indicate clades with dimorphic areoles.

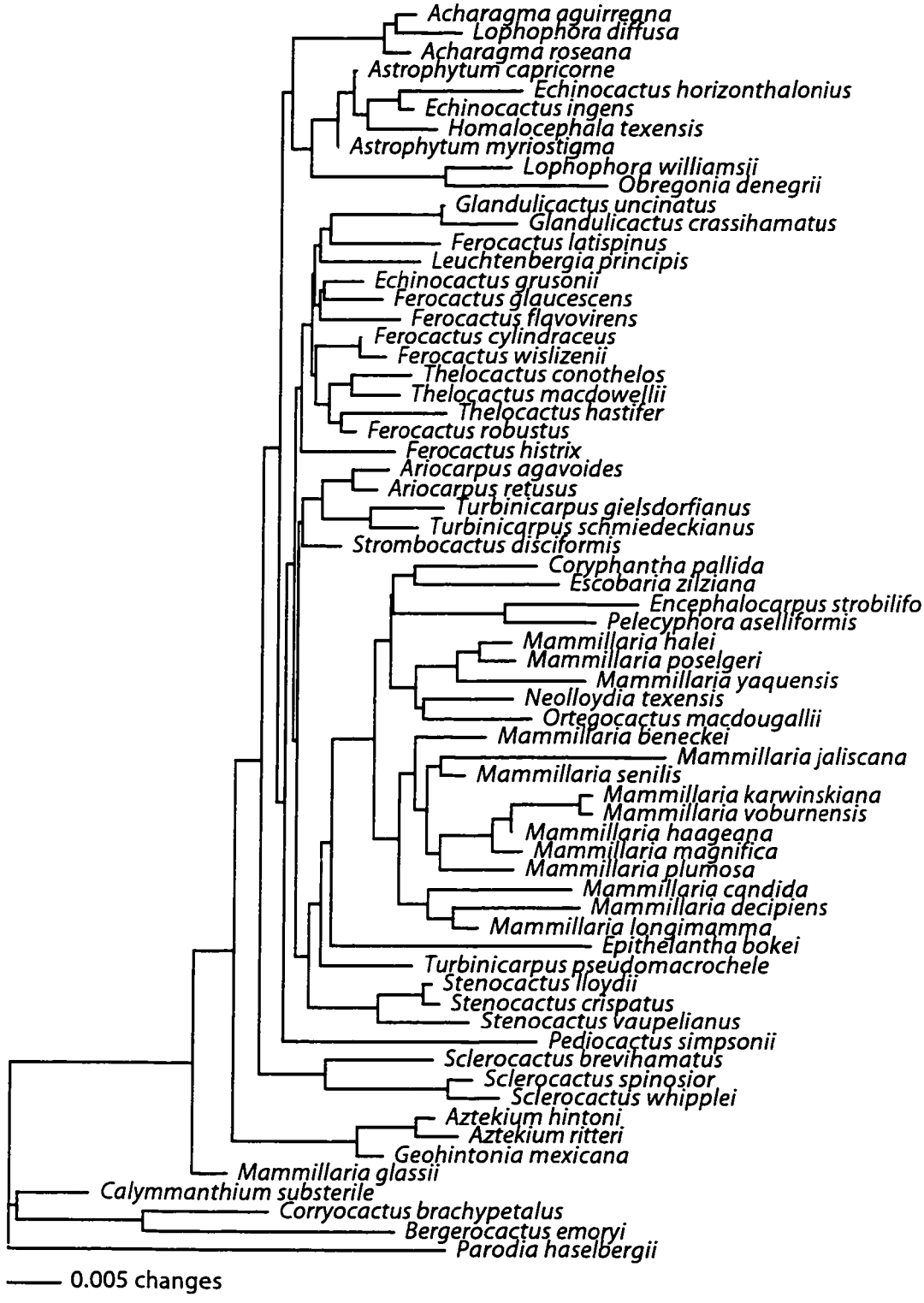

Figure 4-2. Neighbor-joining tree for the tribe Cacteae based on F81 distances.

was shown with *Bergerocactus emoryi* (Pachycereeae), and *Parodia haselbergii* and *Corryocactus brachypetalus*, members of the morphologically similar South American tribe Notocacteae. A number of synapomorphic substitutions resulted in a decay value of 9 with 100% bootstrap support, providing robust support for the Cacteae clade. The monophyly of tribe Cacteae was further tested using *rpl*16 intron sequences in which *Austrocylindropuntia* Backeberg (subfamily Opuntioideae) and *Maihuenia poepegii* (Otto ex Pfeiffer) Philippi ex Schumann (subfamily Maihuenioideae) were used as outgroups for comparisons with each genus of the Cacteae used in this study, together with representatives of all other tribes in the subfamily Cactoideae (Butterworth and Wallace, unpublished data). Support for monophyly of the tribe Cacteae was also very strong (98% bootstrap) with this test.

AZTEKIUM CLADE. The *Aztekium* Clade forms the sister-group to the remaining taxa of the Cacteae. Plants in this clade typically are globose to subglobose, rarely short columnar reaching 20 cm by 10 cm in size. Stem morphology is ribbed, the ribs in *Aztekium* having characteristic transverse wrinkles. Spines are notable by their absence from mature areoles; even in young areoles they are highly reduced and very brittle. *Aztekium* and *Geohintonia* presently are restricted to a small area of eastern Nuevo Leon in NE Mexico. Hunt and Taylor (1992) suggested that *Geohintonia* may represent an intergeneric hybrid involving *Aztekium* and possibly *Echinocactus horizonthalonius*. Corriveau and Coleman (1988) demonstrated biparental inheritance of chloroplast DNA in *Rhipsalidopsis* Britton and Rose, and *Zygocactus* Schumann but maternal inheritance in *Echinocereus* Engelmann and *Opuntia* Miller. If *Geohintonia* is descended from an ancient intergeneric hybrid, and its plastid organelles are maternally inherited, then the maternal parent of the ancient hybrid was closely related to *Aztekium*, probably *A. hintonii* which is sympatric with *Geohintonia*. However, if the chloroplasts of *Geohintonia* show biparental inheritance then *Aztekium* could represent the descendant of either the pollen or ovule donor. The relationship between *Aztekium* and *Strombocactus* Britton and Rose has been cause for discussion. In a preliminary list of accepted genera

by the working party of the International Organization for Succulent Plant Study (IOS) (Hunt and Taylor 1986) and a follow-up report (Hunt and Taylor 1990), the generic status of *Aztekium* was accepted, although among members of the working party, opinion was divided as to whether *Aztekium* and *Strombocactus* were congeneric or convergent. Anderson and Skillman (1984) concluded that *Aztekium* and *Strombocactus* should each be recognized at the generic level, citing a number of differences in vegetative, floral, pollen and seed morphology. The phylogeny presented in this paper strongly supports (bootstrap 100%, decay 7) a clade containing *Aztekium* and *Geohintonia* and does not support a close relationship between *Aztekium* and *Strombocactus*.

ECHINOCACTUS CLADE. The clade comprising *Astrophytum*, *Echinocactus horizonthalonius*, *E. ingens* and *Homalocephala* are globose to shortly columnar cacti with ribbed stems. Areoles are large, and in some species of *Astrophytum*, spines are lacking. Flowers are shortly funnelform to campanulate, the wooly pericarpel having numerous spine-tipped bracts. These cacti are distributed throughout Mexico and SW United States. A close relationship between genera of this clade is also supported by chloroplast restriction-site data (Wallace 1995). Previous authors (Bravo-Hollis and Sánchez-Mejorada 1991; Ferguson 1992; Barthlott and Hunt 1993) have considered *Homalocephala* as congeneric with *Echinocactus*. The *rpl*16 data (this paper) does not fully resolve the relationships between *Astrophytum*, *Echinocactus*, and *Homalocephala* that were shown by Wallace (1995), instead displaying a trichotomy, such that with the inclusion of *Homalocephala*, the genus *Echinocactus* may be paraphyletic. These data also corroborate the conclusion of Cota and Wallace (1997) that *Echinocactus grusonii* is more closely related to members of the genus *Ferocactus* than to other species in the *Echinocactus* clade.

SCLEROCACTUS CLADE. Porter *et al.* (2000) attempted to define generic boundaries for the morphologically diverse genus *Sclerocactus* using chloroplast trnL-trnF sequence data. Although sampling from other genera of the tribe was not as broad as in the study presented

here, sampling from within the genus *Sclerocactus* clearly contradicted the hypothesized close relationship between *Sclerocactus* and *Pediocactus*, suggested by previous authors (Arp 1972 as cited in Porter *et al.* 2000; Benson 1982). Our phylogeny places members of *Sclerocactus* in a well-supported clade (bootstrap 89%, decay 4) and shows no affinity between *Sclerocactus* and the genus *Pediocactus*; thus our results are consistent with those of Porter *et al.* (2000). The working party of the IOS (Hunt and Taylor 1986, 1990) and Barthlott and Hunt (1993) treat the genus *Glandulicactus* as a synonym of *Sclerocactus*. Ferguson (1991 and pers. comm.) disagrees with the placement of *Glandulicactus* within *Sclerocactus*, based on vegetative and floral morphology. Our data support Ferguson's view (see section on *Ferocactus* Clade).

Lophophora Clade. Although there is strong support for this clade (87% bootstrap, 5 decay steps), few morphological features unite this clade. All members have napiform or carrotlike tap-root systems, although these features are also found in other members of the tribe.

The two species of *Acharagma* have been a source of taxonomic confusion. Described originally in the genus *Echinocactus*, *E. roseanus* was transferred into the genus *Gymnocactus* Backeberg by Glass and Foster (1970), who later also described *G. aguirreanus* (Glass and Foster 1972). However, Anderson and Ralston (1978) felt that these two species were better placed in the genus *Turbinicarpus*, contrary to the views of Glass and Foster (1977), who felt that despite high degrees of similarity in distribution, appearance, and flower, fruit and seed morphology, the larger size and generally heavier spination of species of *Gymnocactus* warranted recognition as a separate genus. In a review of *Escobaria*, Taylor (1986) placed *G. roseanus* and *G. aguirreanus* as sole members of the section *Acharagma* of *Escobaria*. Unlike other members of the genus, the axillary areole and tubercular groove is absent in these two species. Furthermore, the flowers are borne in a zone adjacent to the spine-bearing areoles, in contrast to the more typical position in the axils of the tubercles. Glass (1998) elevated Taylor's section *Acharagma* to the rank of genus following Zimmerman's provisional generic treatment in which *Acharagma* was placed in a large clade containing *Ferocactus*, *Coryphan-*

tha, Mammillaria, Ortegocactus, and *Escobaria*, mainly based on foveolate seeds (Zimmerman 1985). However, Zimmerman (1985) acknowledged that *Acharagma* only has weakly derived character states and so his placement of the genus was uncertain. The *rpl*16 intron data suggest the removal of these two species from *Escobaria*, placing them in a well-supported (bootstrap 87%, decay 5) clade containing *Obregonia* and *Lophophora*, the latter shown to be polyphyletic based on this topology.

STROMBOCACTUS. This monotypic genus from the states of Queretero and Hidalgo in central Mexico forms a sister lineage to the "ATEP", *Ferocactus, Stenocactus* and "Mammilloid" clades according to the phylogeny presented in this paper. On the basis of seed morphology, Buxbaum (1958a) suggested that the genus *Strombocactus* ought to include the then monotypic genus *Aztekium*. This was in spite of the tuberculate stem anatomy of *Strombocactus* which contrasts the ribbed anatomy of *Aztekium*. Buxbaum (1958a) explained this by suggesting a progression from the hardened tubercles of *Strombocactus* to the formation of ribs in *Aztekium*. Anderson and Skillman (1984), using morphological and anatomical data, concluded that *Strombocactus* and *Aztekium* each deserved recognition at the genus level. No direct relationship between *Strombocactus* and *Aztekium* is demonstrated in our *rpl*16 phylogeny.

"ATEP" CLADE. This clade's acronym-based name is derived from its included genera — *Ariocarpus, Turbinicarpus, Epithelantha* Weber ex Britton and Rose, and *Pediocactus*, and has poor support in our phylogeny (bootstrap <50%, decay 1 step). Stem morphologies are tuberculate, and in *Ariocarpus* dimorphic areoles are present, this feature an example of convergence with members of the "Mammilloid" clade. *Turbinicarpus* is a genus of around sixteen species of small, inconspicuous cacti from north-central Mexico. Due to poor seed dispersal mechanisms, species of *Turbinicarpus* are highly localized (Glass and Foster 1977). A number of species of *Turbinicarpus* have been allied or subsumed into other genera such as *Gymnocactus* (Backeberg 1970) and *Neolloydia* (Anderson 1986; Hunt and Taylor 1990;

Barthlott and Hunt 1993). However, in the CITES Cactaceae checklist, Hunt chose to accept generic status for species of *Turbinicarpus* leaving only two species in *Neolloydia* (Hunt 1992; 1999). The phylogeny presented here supports the exclusion of *Turbinicarpus* from *Neolloydia* s. str. as *N. conoidea* (type species for the genus) is strongly positioned within the "Mammilloid" clade.

Ferocactus Clade. This clade contains a number of seemingly disparate genera with few morphological affinities (such as conspicuous pericarpel scales) that unite the entire clade. Members of the genus *Ferocactus* possess a number of morphological synapomorphies including nectar-secreting areolar glands and a ring of hairs that separate the stamens from the tepals. Although morphologically striking due to elongate, glaucous tubercles, the single species of *Leuchtenbergia—L. principis* (the "Agave Cactus") is placed in the *Ferocactus* clade. Barthlott and Hunt (1993) describe the flowers of this species as similar to those of *Ferocactus*, and the fruit as being typical of those in subgenus *Ferocactus*—dry, globose to oblong with thick-walls and dehiscing at the base. A close affinity between *Ferocactus* and *Leuchtenbergia* is also demonstrated by the ease with which these genera hybridize. The phylogeny presented here, as well as chloroplast restriction site data (Cota 1997; Cota and Wallace 1997), shows that *Echinocactus grusonii* is more closely related to members of *Ferocactus* (particularly *F. histrix* and *F. glaucescens*) than it is to the remaining species of *Echinocactus* sampled. These species share a number of distinct character traits, including straight or slightly curved, terete central spines as opposed to hooked spines with flat cross-sections that are more typical of *Ferocactus*. In our phylogeny, however, *F. histrix* is positioned outside the *Ferocactus* clade. If *F. histrix* is moved and placed sister to *F. glaucescens* and *E. grusonii*, tree-length increases by only three steps. The slight change in tree-length and low decay value (decay = 1) for the branch separating *F. histrix* from the *Ferocactus* clade implies possible homoplasy in our dataset. Previous molecular studies of *Ferocactus* by Cota and Wallace (1997) and Cota (1997) were only able to partially resolve species relationships between *Ferocactus* and its allies, but did recover similar clades within the genus *Ferocactus*.

Glandulicactus uncinatus and *G. crassihamatus* currently are recognized as *Sclerocactus uncinatus* and *S. uncinatus* ssp. *crassihamatus*, respectively, by a number of authors (Hunt 1992; Barthlott and Hunt 1993; Hunt 1999; Anderson 2001). However, Ferguson (1991) argued that this genus did not belong in *Sclerocactus*, citing a number of morphological differences. Instead, he allied members of this genus with *Ferocactus, Thelocactus*, and *Leuchtenbergia* based on vegetative and floral morphology. Although the phylogeny presented in this paper does not necessarily support Ferguson's viewpoint that *Glandulicactus* should be recognized at genus level, it does corroborate his conclusions that the members of this genus are more closely related to *Ferocactus* and *Thelocactus* than to *Sclerocactus*.

Stenocactus Clade. Comprising about 10 species, *Stenocactus* tends to be separable from the related *Ferocactus* clade by two morphological characters: 1) narrow, fin-like ribs as opposed to wide ribs, and 2) areoles in which the large spines are subtended by the smaller spines as opposed to areoles in which the larger spines subtend the smaller spines in *Ferocactus*. However, Taylor (1983) argued that despite these morphological differences, flower, fruit and seed morphology required a broader generic concept that included the members of *Stenocactus* in the genus *Ferocactus*. Our *rpl*16 phylogeny suggests that the *Stenocactus* clade (bootstrap 100%, decay 4) is distinct from the *Ferocactus* clade.

"Mammilloid" Clade. Although support for the "Mammilloid" clade is not particularly strong (bootstrap 60%, decay 3), members share the morphological synapomorphies of tuberculate stem anatomy and dimorphic areoles (the spine-bearing areoles being apical and the flowering areoles being axillary to the tubercles). Within the clade, generic delimitations have traditionally been confused. *Pelecyphora* and *Encephalocarpus* form a well-supported clade (bootstrap 100%, decay 8). These genera have been treated as congeneric (*Pelecyphora*) by some previous authors (Anderson and Boke 1969; Barthlott and Hunt 1993; Anderson 2001), and are recognized as such in the CITES Cactaceae Checklist (Hunt 1992, 1999). Relationships between *Escobaria* and *Coryphantha* are controversial. Berger (1929 cited in

Zimmerman 1985) subsumed *Escobaria* into *Coryphantha.* Taylor (1986) cites a number of character traits that distinguish the two genera, including pitted seeds and ciliate outer perianth segments in *Escobaria* versus non-pitted seeds and non-ciliate outer perianth segments in *Coryphantha.* Taylor (1986) suggests that *Escobaria* is more closely related to *Mammillaria* than it is to *Coryphantha.* Indeed, a sister relationship between *Escobaria* and *Coryphantha* is suggested by *rpl*16 intron data, although additional data and more intense sampling are required in order to evaluate more robustly their relationships to *Mammillaria.*

Based on *rpl*16 sequences, *Mammillaria* is not monophyletic as currently circumscribed due to the placement of *Neolloydia conoidea* and *Ortegocactus macdougallii.* The working party of the IOS (Hunt and Taylor 1986, 1990) chose to include the genus *Turbinicarpus* and *Gymnocactus* within *Neolloydia.* Barthlott and Hunt (1993) followed the same treatment but suggested that the dimorphic areoles and hence axillary flowers of the type species of *Neolloydia* (*N. conoidea*) were sufficient to justify recognition of *Turbinicarpus* at genus level while continuing to recognize the genus *Neolloydia.* Zimmerman (1985) concludes that *Neolloydia* is distinct from *Coryphantha* and its allies (being more closely related to *Ariocarpus, Obregonia, Lophophora, Strombocactus,* and *Aztekium*), and that the tubercular groove, in this case, is non-homologous with the areolar groove in *Escobaria* and *Coryphantha.* However, Zimmerman failed to cite which species of *Neolloydia* were used in his study, so it is unsure if he used the type—*N. conoidea* or other species referable to *Turbinicarpus.* Our data supports the view of Barthlott and Hunt (1993) that *Neolloydia* in the strict sense (*N. conoidea* and *N. matahuelensis* Backeberg) are not closely related to either *Turbinicarpus* or *Gymnocactus.*

Ortegocactus is a monotypic genus, known only from the state of Oaxaca, Mexico. Although it shares many morphological features with members of the "Mammilloid" clade, taxonomists have had difficulty assessing relationships of this species to other members of the clade. Bravo-Hollis and Sánchez-Mejorada (1991) placed *Ortegocactus* in the genus

Neobesseya Britton and Rose. Zimmerman (1985) concluded that *Ortegocactus* was a member of a phylogenetically distinct clade containing *Coryphantha, Escobaria* and *Mammillaria.* The *rpl*16 phylogeny suggests that *Ortegocactus* is more closely related to *Mammillaria* than to other members of the "Mammilloid" clade, and shows no direct relationship with *Coryphantha* or *Escobaria.*

The clade containing *M. halei, M. poselgeri,* and *M. yaquensis* is phylogenetically distinct (bootstrap 83%, decay 1) from the remaining species sampled in *Mammillaria* sensu stricto. Two of these three species (*M. halei* and *M. poselgeri*) are referable to the genus *Cochemiea. Mammillaria yaquensis* (series *Ancistracanthae* Schumann) has been allied to other members of *Cochemiea* by Lüthy (1995). That *Cochemiea* (represented by *M. halei* and *M. poselgeri* in this study) are found only in Baja California raises the question of their origin and dispersal from mainland Mexico. The series *Ancistracanthae* are distributed in western Mexico and Baja California reaching as far north as the southern USA (California Arizona and New Mexico). A reasonable hypothesis suggests that ancestral members of the *Ancistracanthae* migrated northwards in mainland Mexico, before migrating south through Baja California. The geologic history of Baja California seems unclear, but there is evidence that the Gulf of California began to separate around 4.5 million yr ago (Atwater 1970) or 5.5 million yr ago according to Riddle *et al.* (1997). However, the gulf may have been in existence for the last 12 million yr (Gastil *et al.* 1983). Assuming a north-south migration of ancestral *Ancistracanthae*, the phylogeny presented here suggests a more recent origin for *Cochemiea.* Hunt (pers. comm.) disputes recognizing *Cochemiea* as distinct, stating that ornithophilous flowers (found only in *Cochemiea*) are derived and contradict a sister-group relationship to other members of *Mammillaria.* Our phylogeny does support ornithophily as being derived and suggests that *Cochemiea* arose from an *Ancistracanthae*-like ancestor.

Within the main clade of *Mammillaria*, there are a number of species whose inclusion in the genus has been disputed by various cactus taxonomists. *Mammillaria beneckei* was

considered by Buxbaum (1951, in Hunt 1977a) as a distinct genus (*Oehmea*) and argued that this was an example of morphological convergence with *Mammillaria*, but that it was actually derived from a *Thelocactus*-type ancestor. Hunt (1977a) subsumed the genus *Oehmea* within *Mammillaria*, giving it subgeneric status based upon the rugose/pitted seed testa, which is also found in other members of the genus. In their work on the Cactaceae, Britton and Rose (1919–1923) gave separate generic status to *Mammillaria senilis* by describing it within the genus *Mamillopsis*. Their justifications for this were based on a number of floral traits that they considered sufficiently different from *Mammillaria* to warrant its generic status. However, Hunt (1971) concluded that vegetative, floral, and seed morphology when taken in the sum of their characters did not support generic status of *Mamillopsis* and that it should only be retained at the subgeneric level within *Mammillaria*. Britton and Rose (1919–1923) also elevated Schumann's (1899) subgenus *Dolichothele* (represented in this study by *M. longimamma* DC) to genus level, separating it from other species of *Mammillaria* due to its very elongate tubercles. As with *Mamillopsis*, Hunt (1971) believed that there were insufficient differences to justify *Dolichothele* at the rank of genus, instead accepting it as a subgenus of *Mammillaria*. Our *rpl*16 phylogeny does not support the view of Buxbaum (1951, in Hunt 1977a) regarding the generic status of *Oehmea* because no direct relationship between *Oehmea* and *Thelocactus* is demonstrated. Although sampling from the genus *Mammillaria* is limited, our data also suggest that recognition of *Mamillopsis* and *Dolichothele* at the generic rank is unwarranted as they are nested within other *Mammillaria* species. Further sampling from *Mammillaria* is required and for the present, *Oehmea*, *Mamillopsis*, and *Dolichothele* should be retained in *Mammillaria*.

The relationship of *Mammillaria candida* to other members of the genus has also been a source of past debate. Schumann (1899) considered this species to be within his subgenus *Eumamillaria* (true *mammillarias*). Buxbaum (1951, in Hunt 1977a) elevated this species to genus status—*Mammilloydia candida* based solely on a tuberculate seed testa morphol-

ogy. Riha and Riha (1975) disputed Buxbaum's observations, going so far as to suggest that Buxbaum had accidentally observed seed material that was not from *Mammillaria candida*. Hunt (1977) argued in support of Buxbaum, suggesting that *Mammilloydia candida* was the product of a separate evolutionary lineage than that of the remaining species of *Mammillaria*. However, he considered the retention of subgenus *Mammilloydia* a taxonomic compromise. The International Cactaceae Systematics Group recently has accepted that *M. candida* merits recognition at the generic rank as *Mammilloydia* (Hunt 1999) and it is treated as such in Anderson (2001). Sequence analysis of the *rpl*16 intron presented here, and from a more broad sampling of the "Mammilloid" clade (Butterworth 2000) indicate that recognition of *M. candida* at the rank of genus would render *Mammillaria* paraphyletic. Further studies on the "Mammilloid" clade are in progress to resolve these issues.

Morphological Evolution

EVOLUTION OF TUBERCLES IN THE CACTEAE. Buxbaum (1958a) presented a number of different scenarios in which tubercular stem morphologies may have arisen in the Cactaceae. Within the tribe Cacteae, he described the convergent evolution (in a number of lineages within the tribe) of transversely-arranged tubercles formed from ribs in which the basal portions of the podaria have become enlarged to form tubercles, implying that a ribbed stem morphology represents the primitive condition for the tribe. Gibson and Nobel (1986) also suggest that the primitive condition for the subfamily Cactoideae is likely based on ribbed-stem morphology. From our studies using *rpl*16 intron sequence data, it appears that in the Cacteae tubercular stem morphologies represent a derived condition. The question of multiple origins of tubercles or reversals to ribbed stems is debatable. The most parsimonious explanation based on the phylogeny presented in this paper is that tubercular stems have arisen independently in a number of clades, once following the divergence between the *Echinocactus* clade and the remaining Cacteae. A reversal to ribbed stems is implicated in *Ferocactus histrix*,

Stenocactus, and the *Ferocactus* clade, with secondarily derived tubercular stem morphologies representing a zone of transition between ribs and tubercles. The genus *Ferocactus* has ribbed stems, while *Leuchtenbergia* has very distinct elongate tubercles. *Glandulicactus* has deeply notched ribs and may represent the intermediate condition, and in *Thelocactus*, both ribs and tubercles are present—*Thelocactus hastifer* with distinct, spiraling ribs divided into tubercles, and the sister clade of *T. conothelos* and *T. macdowellii* having indistinct ribs and pronounced tubercles (Anderson 1987). There is also a switch from ribbed to tubercular stems in the "Mammilloid" Clade, whose members also share the synapomorphy of having dimorphic areoles (see below).

EVOLUTION OF DIMORPHIC AREOLES. The majority of genera in the Cacteae produce flowers from the spine-forming areoles. However, a number of taxa in the tribe have dimorphic areoles in which spines and flowers are borne from different regions or even from separate areoles. To an extent this correlates with tubercular stem morphologies where spines are produced from apical areoles (the axillary areoles becoming reproductive). Buxbaum (1958a) proposed that the evolution of dimorphic areoles in the tribe Cacteae occurred along two distinct lines. The first lineage shows a succession from *Leuchtenbergia*, which has elongated tubercles tipped by undifferentiated areoles, to *Roseocactus* (*Ariocarpus fissuratus, A. kotschoubeyanus*) with areoles forming an elongated furrow along the length of the tubercle with separate floral and spine-bearing regions (Anderson 1960), to *Ariocarpus* with separate floral and spine-bearing areoles and some species lacking spine-bearing areoles altogether (Anderson 1960). Buxbaum's second evolutionary lineage occurred from a non-differentiated "*Thelocactus*-type" areole in which growth occurs below the areole causing it to be forced to the tip of the tubercle. In a number of species, lengthening growth divides the growing point forcing the spine producing part towards the tubercle tip, the flower producing region remains in the tubercle axil. Species with this form of dimorphic areole may have a groove running along the adaxial length of the tubercle connecting the vegetative and reproductive

areoles. In *Mammillaria*, the groove is absent due to division of the growing point at a very early stage in development. According to our phylogeny, undifferentiated areoles represent the plesiomorphic condition for the Cacteae with the evolution of dimorphic areoles occurring independently in *Ariocarpus* and the "Mammilloid" clade.

In summary, the pattern of evolution that we present in the tribe Cacteae, as inferred from the *rpl*16 intron phylogeny, suggests that the *Aztekium-Geohintonia* clade represents a relictual, yet highly specialized lineage. The remaining members of this North American barrel cactus tribe have undergone diversification into several clades, the more derived clades manifesting a shift from plesiomorphic ribbed stems to those that are tuberculate, concomitantly undergoing a general reduction in plant size. Shifts in floral position and areole are also inferred from the phylogeny. These changes occurred in parallel within the Cacteae, further adding to the systematic confusion experienced by many earlier cactologists. Although the inferred evolutionary relationships we present are based on data from a single molecular marker of the plastid genome, the resulting tree topology and clades defined are telling as to the broad-scale, intergeneric relationships within the tribe that have heretofore only received "support" through speculative conclusions, accompanied by little empirical analyses.

Here we broadly sample representative members of the tribe Cacteae in a uniformly comparative fashion, and evaluate the group to determine its primary lineages. The intergeneric relationships inferred now lend themselves to further testing with additional markers and more intensive sampling for the more species-rich genera (e.g., *Mammillaria, Coryphantha, Escobaria, Ferocactus*), as well as reexamining those clades that were not well supported (e.g., the "ATEP" Clade). These investigations are ongoing at present and will extend the value of the present study through the use of its conclusions in prudent outgroup sampling, identification of morphological evolutionary trends among the taxa, and by establishing a baseline phylogeny for integration with other similar studies being conducted on other tribes in the Cactaceae.

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CHAPTER 5

Molecular Phylogenetics of *Mammillaria* (Cactaceae) Using Non-Coding Chloroplast DNA Sequence Variation

A paper to be submitted to Systematic Botany

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INTRODUCTION

Following the re-organization of the genus *Opuntia* Miller by Wallace and Dickie (2002) into a number of segregate genera, the genus *Mammillaria* Haworth has taken precedence as the most species rich genus in the cactus family. Modern estimates of species numbers vary greatly depending upon circumscription at both the generic and specific levels. 181 species are recognized by Pilbeam (1999) while Hunt (1999) accepts 145 species.

Members of the genus *Mammillaria* are typically viewed as being low growing globular cacti with a distinctly tuberculate stem morphology. Plants may either be solitary or form massive mounds. These traits are shared with other members of the 'Mammilloid Clade' (Butterworth *et al.*, 2002), which also share the presence of dimorphic areoles – the vegetative (spine-bearing) areole being borne on the tubercle apex while the flowering areoles are located in the axils of the tubercles. *Mammillaria* is distinct from these other genera (*Coryphantha, Escobaria, Pelecyphora, Neolloydia* and *Ortegocactus*) in lacking an adaxial groove running from the vegetative areole, in some cases along the entire length of the tubercle. Distribution of the genus ranges from Venezuela and Colombia in the south to the SW United States with maximal diversity and species richness in Mexico.

Although used by Linnaeus as type species for the genus *Cactus* (Linnaeus, 1753), *Cactus mammillaris* L. was transferred to, and designated type species (as *M. simplex*) of the genus *Mammillaria* by Haworth (1812). The name *Mammillaria* as described by Haworth is a later homonym, the name first being used to describe a genus of algae by Stackhouse in 1809.

In 1930, the name *Mammillaria* was conserved for the cactus genus during the International Botanical Congress of that year.

Pfeiffer (1837) introduced the first infrageneric division of *Mammillaria*. This classification divided the genus into two groups based upon spine characteristics, and was followed in 1845 by a more complex classification by Salm-Dyck (1845) which recognized eight groups just below the rank of genus. Both these early classifications of *Mammillaria* were broadly circumscribed and in 1856, George Engelmann, a St. Louis physician, laid the groundwork for future splitting of the genus into segregate genera. Engelmann (1856) explicitly recognized and described two subgenera in *Mammillaria*. Members of subgenus *Coryphantha* included species with grooved tubercles and flowers produced from the current year's growth, whereas the species in subgenus *Eumammillaria* had ungrooved tubercles and flowers were produced from tubercles of the previous year.

In 1898, Schumann published a comprehensive work on the cactus family (Schumann, 1898). Although he included within *Mammillaria* members of the genus *Coryphantha* (as subgenus *Coryphantha*), Schumann recognized three other subgenera – *Dolichothele* Schumann, *Cochemiea* (Brandegee) Schumann, and *Eumamillaria*. Even though previous authors (Pfeiffer, 1837; Salm-Dyck, 1845) had described infrageneric taxa above the level of species in *Mammillaria*, Schumann explicitly named the specific infrageneric ranks of section and series. Both subgenera *Dolichothele* and *Cochemiea* included a single series each, however, subgenus *Eumamillaria* was further divided into sections *Hydrochylus* Schumann and section *Galactochylus* Schumann depending upon whether the members had watery or milky sap respectively. Section *Hydrochylus* was further split into six series and section *Galactochylus* into five series.

Since Schumann's work on *Mammillaria*, a number of subsequent authors have held differing opinions regarding generic delimitations in *Mammillaria*. Britton and Rose (1922; 1923) recognized only a narrow circumscription of *Mammillaria*, splitting Schumann's view

of the genus into nine genera. Contrary to Britton and Rose, Berger (1929) took a slightly broader view of *Mammillaria* and recognized many of the infrageneric taxa of Schumann.

Buxbaum (1951c) believed that *Mammillaria* was not monophyletic, stating that there was a '*Mammillaria* Stage' in the evolution of North American barrel cacti (tribe Cacteae) in which plants had the appearance of members of *Mammillaria*. Furthermore, the '*Mammillaria* Stage' had been reached in a number of independent lineages. During the following years, Buxbaum modified his infrageneric and generic delimitations of *Mammillaria* and closely related taxa (Buxbaum, 1951b, 1954, 1956a; 1956b) resulting in a treatment that took a narrow circumscription of *Mammillaria* and recognized a number of segregate genera. However, when Moran (1953) proposed reunifying Buxbaum's segregate genera with *Mammillaria* for Hortus Third, Buxbaum relented, accepting a much broader circumscription of the genus *Mammillaria* (1956a; 1956b).

Two later authors have attempted to produce up-to-date classifications of *Mammillaria*. David Hunt, working in the 1960's and 1970's attempted to bring together the work of Schumann (1898) and Buxbaum (1951b; 1951c; 1954; 1956a; 1956b) into a simple infrageneric classification. Hunt (Hunt, 1971, 1977a, b, c, 1981) did not hesitate in recognizing the genus *Coryphantha* as being clearly separate from *Mammillaria*. Within the genus *Mammillaria*, Hunt recognized five subgenera – *Mammilloydia* (Buxb.) Moran, *Oehmea* (Buxb.) Hunt, *Dolichothele*, *Cochemiea* Brand., *Mamillopsis* Morren ex B. & R., and *Mammillaria*. Of these subgenera, only subgenus *Mammillaria* was divided further, being split into three sections, which were modified from Schumann's (1898) sections *Hydrochylus* (divided into *Hydrochylus* and *Subhydrochylus* Backeberg ex Hunt) and *Galactochylus* (as section *Mammillaria*). Hunt further recognized a number of series within the sections of subgenus *Mammillaria*.

Lüthy (1995) took a phenetic approach to the classification of *Mammillaria* and undertook a detailed morphological analysis of the genus. This data, supplemented with biochemical and ecological data, was used to infer relationships in the genus and produce a classification that was independent of past taxonomic treatments of the genus. Lüthy took

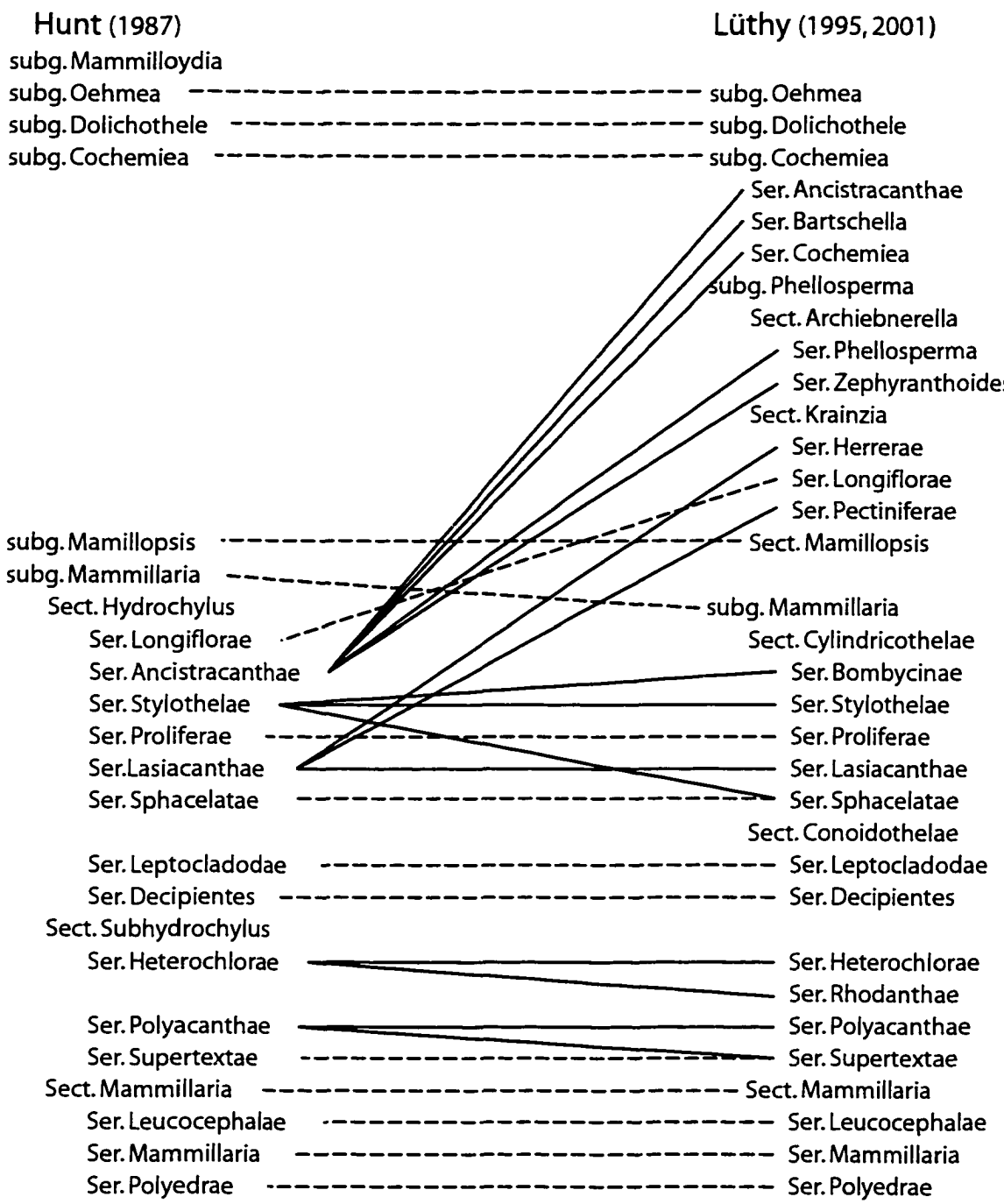


Figure 5-1. Comparison of Hunt's (1987) infrageneric classification of *Mammillaria* with that of Lüthy (1995, 2001). subg. = subgenus; Sect. = section; Ser. = series. The dashed lines indicate infrageneric groupings with similar circumscriptions between the two classifications, solid lines show circumscriptional differences between the two classifications.

a fairly narrow circumscription of *Mammillaria*, preferring to recognize *Coryphantha* and *Mammilloydia* Buxbaum as distinct from *Mammillaria*. The classification produced by Lüthy includes four subgenera, four sections, and twenty-two series.

The infrageneric classifications of Hunt (1981) and Lüthy (1995) show a number of significant differences (see Figure 5-1) and represent the endpoints of different approaches in taxonomic inference. In the last two decades, the use of molecular sequence data in cladistic studies have had a significant impact on the world of taxonomy and systematics. Such methods provide a unique way of investigating taxonomic problems such as addressing the differences between Hunt and Lüthy. The aim of this paper is to use molecular phylogenetic techniques (namely sequence data from the *rpl*16 intron and *psb*A-*trn*H Intergenic Spacer regions of the chloroplast) to investigate cladistic relationships and classifications in the genus *Mammillaria* and attempt to resolve the differences in past infrageneric classifications of the genus.

Materials and Methods

Taxonomic Sampling

A total of 127 taxa were sampled (Table 5-1) including 115 representative taxa from *Mammillaria*. Other members from the 'Mammilloid Clade' (Butterworth *et al.*, 2002) included individual taxa from *Ortegocactus*, *Pelecyphora* and *Neolloydia*, four taxa from *Escobaria*, and three taxa from *Coryphantha*. Selected outgroup taxa for the study were *Ferocactus robustus* and *Stenocactus multicostatus*.

Table 5-1. Taxa sampled for the *rpl16* intron and *psbA-trnH IGS* study. DES = Desert Botanical Garden, Arizona; HUMO = Universidad Autónoma del Estado de Morelos, Mexico; ISC = Ada Hayden Herbarium, Iowa State University; UCONN = University of Connecticut.

Taxon	Source/Voucher	GenBank No.
Coryphantha durangensis (Schumann) Britton & Rose	HNT 52667	
Coryphantha elephantidens (Lemaire) Lemaire	DES 1955-5362-0101	
Coryphantha pallida Britton & Rose	Hugo Cota 8050—HUMO	
Escobaria chihuahuensis Britton & Rose	DES 1973-0264-0202	
Escobaria hesteri (Y. Wright) Buxbaum	DES 1985-0505-0105	
Escobaria tuberculosa (Engelmann) Britton & Rose	DES 1986-0619-0101	
Escobaria zilziana (Boedecker) Backeberg	DES 1989-0137-0102—DES	
Ferocactus robustus (Pfeiffer) Britton & Rose	H. Cota 8045—HUMO	
Mammillaria albicans (Britton & Rose) Berger	Mesa Garden 555.2	
Mammillaria albilanata Backeberg	HNT 72568	
Mammillaria anniana Glass & Foster	W.A. Fitz Maurice 2193—DES	
Mammillaria armillata K. Brandegee	Mesa Garden 556.32	
Mammillaria bachmanii Boedeker ex Berger	ex. Hort. Miles To Go Nursery	
Mammillaria backebergiana F. G. Buchenau	HNT 41878	
Mammillaria barbata Engelmann	Mesa Garden 563.4	
Mammillaria beneckei Ehrenberg	DES 1993-0550-0101—DES	
Mammillaria blossfeldiana Boedeker	Mesa Garden 572.3	
Mammillaria bocasana Poselger	W.A. Fitz Maurice 1916—DES	
Mammillaria bombycina Quehl	W.A. Fitz Maurice 1821—DES	
Mammillaria boolii Lindsay	Mesa Garden 582	
Mammillaria brachytrichion Luethy	W.A. Fitz Maurice 2358—DES	
Mammillaria cadereytensis Craig	Mesa Garden 584	
Mammillaria candida Schweidweiler	DES 1957-5907-0101—ISC	
Mammillaria capensis (Gates) Craig	Mesa Garden 594.5	
Mammillaria carmenae Castaneda & Nunez	ex Hort. UCONN	
Mammillaria carnea Zuccarini ex Pfeiffer	Mesa Garden 597.5	
Mammillaria cerralboa Britton & Rose (Orcutt)	Mesa Garden 602.2	
Mammillaria crinita De Candolle	W.A. Fitz Maurice 2153—DES	
Mammillaria crinita De Candolle	W.A. Fitz Maurice 2346—DES	
Mammillaria crinita De Candolle	W.A. Fitz Maurice 2294—DES	
Mammillaria crinita ssp. *scheinvariana* (Ortega-Varela & Glass) W.A. & B. Fitz Maurice	W.A. Fitz Maurice 2378—DES	
Mammillaria decipiens Scheidweiler	HNT 68830—ISC	
Mammillaria dioica K. Brandegee	Steven Brack 1249	
Mammillaria discolor Haworth	623	
Mammillaria dixanthocentron Backeberg ex Mottram	Mesa Garden 624.84	
Mammillaria duoformis Craig & Dawson	HNT 49132	
Mammillaria duwei Rogozinski & Appenzeller	W.A. Fitz Maurice 1641—DES	
Mammillaria elongata De Candolle	F. Otero 045	
Mammillaria erythrosperma Boedeker	W.A. Fitz Maurice 1766—DES	
Mammillaria fittkaui Glass & Foster	W.A. Fitz Maurice 2107A—DES	
Mammillaria formosa Galeotti ex Scheidweiler	Mesa Garden 644.5	
Mammillaria fraileana (Britton & Rose) Boedecker	Mesa Garden 646.52	
Mammillaria gasseriana Boedeker	W.A. Fitz Maurice 2289—DES	
Mammillaria geminispina Haworth	4/4/76 CP6	
Mammillaria glassii Foster	HNT 60162—ISC	
Mammillaria goodridgei Scheer ex Salm-Dyck	Mesa Garden 660	
Mammillaria vetula ssp. *gracilis* (Pfeiffer) Hunt	ex. Hort ISC	
Mammillaria grahamii Engelmann	Mesa Garden 665.4	
Mammillaria grusonii Runge	Mesa Garden 669.1	
Mammillaria guelzowiana Werdermann	Mesa Garden 671.1	
Mammillaria haageana Pfeiffer	H. Cota 8053—HUMO	
Mammillaria halei T. Brandegee	HNT 72646—ISC	
Mammillaria hernandezii Glass & Foster	s.n.	
Mammillaria herrerae Werdermann	HNT 75886	
Mammillaria huitzilopochtlii Hunt	s.n.	
Mammillaria humboldtii Ehrenberg	Mesa Garden 702.2	
Mammillaria hutchinsoniana (Gates) Boedeker	Mesa Garden 705.3	

Table 5-1 continued.

Taxon	Source/Voucher	GenBank No.
Mammillaria insularis Gates	Mesa Garden 707.2	
Mammillaria jaliscana (Britton & Rose) Boedeker	W.A. Fitz Maurice 1817—DES	
Mammillaria karwinskiana Martius	H. Cota s.n. —ISC	
Mammillaria klissingiana Boedeker	Mesa Garden 714	
Mammillaria lasiacantha Engelmann	HNT 28268	
Mammillaria crinita ssp. *leucantha* (Boedeker) Hunt	W.A. Fitz Maurice 2199—DES	
Mammillaria limonensis Reppenhagen	W.A. Fitz Maurice 2222—DES	
Mammillaria lindsayi Craig	HNT 39258	
Mammillaria longimamma De Candolle	DES 1992-0049-0203—DES	
Mammillaria luethyi G. S. Hinton	s.n.	
Mammillaria magnifica Buchenau	ex Hort. HNT—ISC	
Mammillaria magnimamma Haworth	HNT 52528	
Mammillaria mainiae K. Brandegee	Mesa Garden 750.8	
Mammillaria mammillaris (L.) Karsten	754?	
Mammillaria marcosii W.A. & B. Fitz Maurice & Glass	W.A. Fitz Maurice 2364—DES	
Mammillaria mathilde Glass & Foster	W.A. Fitz Maurice 1647—DES	
Mammillaria mazatlanensis Schumann ex Guerke	DES 1986-0542-10	
Mammillaria melanocentra ssp. *rubrograndis* (Reppenhagen & Lau) Hunt	HNT 46472A	
Mammillaria mercadensis Patoni	W.A. Fitz Maurice 2332—DES	
Mammillaria mercadensis Patoni	W.A. Fitz Maurice 2344—DES	
Mammillaria microhelia Werdermann	Mesa Garden 785.2	
Mammillaria moelleriana Boedeker	W.A. Fitz Maurice 2336—DES	
Mammillaria multidigitata Radley ex Lindsay	Mesa Garden 799	
Mammillaria mystax Martius	Mesa Garden 802.4	
Mammillaria nana Backeberg ex Mottram	W.A. Fitz Maurice 1980—DES	
Mammillaria nazasensis (Glass & Foster) Reppenhagen	W.A. Fitz Maurice 2323—DES	
Mammillaria neopalmeri Craig	Mesa Garden 807.5	
Mammillaria oteroi Glass & Foster	HNT 47698	
Mammillaria parkinsonii Ehrenberg	C. Jaromir 303	
Mammillaria patonii (Bravo) Boedeker	ex. UCONN 2218	
Mammillaria pectinifera Weber	F. Otero 215	
Mammillaria peninsularis (Britton & Rose) Orcutt	HNT 74523	
Mammillaria pennispinosa Krainz	W.A. Fitz Maurice 2273—DES	
Mammillaria perezdelarosae Bravo & Scheinvar	W.A. Fitz Maurice 1644—DES	
Mammillaria petrophila ssp. *baxteriana* (Gates) Hunt	s.n.	
Mammillaria phitauiana (Baxter) Werdermann ex Backeberg	HNT 36954	
Mammillaria picta Meinshausen	Mesa Garden 838	
Mammillaria plumosa Weber	HNT 28166—ISC	
Mammillaria polyedra Martius	DES 1939-0234-01	
Mammillaria polythele Martius	s.n.	
Mammillaria pondii ssp. *setispina* (Coulter) Hunt	Mesa Garden 896	
Mammillaria poselgeri Hildmann	DES 1983-0746-1018—ISC	
Mammillaria pottsii Scheer ex Salm-Dyck	Mesa Garden 852	
Mammillaria prolifera (Miller) Haworth	Mesa Garden 858.2	
Mammillaria rekoi (Britton & Rose) Vaupel	Mesa Garden 865	
Mammillaria rettigiana Boedeker	W.A. Fitz Maurice 2091—DES	
Mammillaria rhodantha Link & Otto	DES 1966-8392-01	
Mammillaria schumannii Hildmann	Mesa Garden 885.57	
Mammillaria schwarzii Shurly	W.A. Fitz Maurice 1687B—DES	
Mammillaria senilis Loddiges ex Salm-Dyck	Mesa Garden s.n.—ISC	
Mammillaria sinistrohamata Boedeker	W.A. Fitz Maurice 2316—DES	
Mammillaria sphacelata Martius	HNT 45363	
Mammillaria spinosissima Lemaire	HNT 53663	
Mammillaria stella-de-tacubaya Heese	W.A. Fitz Maurice 2322—DES	
Mammillaria supertexta Martius ex Pfeiffer	Mesa Garden 917.76	
Mammillaria tezontle Fitz Maurice	W.A. Fitz Maurice 1983—DES	
Mammillaria thornberi Orcutt	Mesa Garden 926	
Mammillaria thornberi ssp. *yaquensis* (Craig) Hunt	HNT 7715—ISC	
Mammillaria tonalensis Hunt	Mesa Garden 928.5	

Table 5-1 continued.

Taxon	Source/Voucher	GenBank No.
Mammillaria voburnensis Scheer	Lippold s.n. —UCONN	
Mammillaria voburnensis ssp. *eichlamii* (Quehl) Hunt	HNT 78326	
Mammillaria weingartiana Boedeker	W.A. Fitz Maurice 1544—DES	
Mammillaria wildii Dietrich	W.A. Fitz Maurice 2190—DES	
Mammillaria wrightii Engelmann	S. Brack 210	
Mammillaria zacatecasensis Shurly	W.A. Fitz Maurice 2020—DES	
Mammillaria zeilmanniana Boedeker	W.A. Fitz Maurice 1764—DES	
Mammillaria zephyranthoides Scheidweiler	Mesa Garden 962	
Neolloydia conoidea (De Candolle) Britton & Rose	S. Brack 1888—ISC	
Ortegocactus macdougallii Alexander	R. Wallace s.n.—ISC	
Pelecyphora aselliformis Ehrenberg	DES 1961-6848-0101—DES	
Stenocactus multicostatus (Hildmann ex Schumann) Berger ex Hill	ex Hort—UCONN	

DNA Extraction and Purification

Extractions of total genomic DNA of representative taxa were carried out using one of three methods:

1. Modified organelle pellet method suitable for mucilaginous material. DNA was extracted from despined, green plant material according to previously published methods (Butterworth et al., 2002; Wallace, 1995; Wallace and Cota, 1996), and the DNA pellet was resuspended in 1ml of TE.

2. Nucleon Phytopure™ plant and fungal kit for 1g samples (Amersham Life Science). Extracted DNA was resuspended in 1ml TE and stored at -20°C.

3. DNEasy Plant Mini kit (Qiagen). Approximately 90mg of green plant material was used for each extraction. The manufacturer's protocol was followed with the exception that the DNA was eluted in 50μL of sterile distilled water.

Amplification and Sequencing

Double-stranded amplification of the target sequences was done using the Polymerase Chain Reaction (PCR) conducted in a MJ Research PTC-100 thermal cycler. Primer sequences of amplification and sequencing primers are shown in Table 5-2.

Table 5-2. List of primers and references used in this study. The primer PsbAF is modified (shown in underlined bold) from the primer given in Sang *et al* (1997). Primers F71 and R1661 were used for amplification only, R1516, R637 and F543 were used for sequencing.

Gene	Primer Name	Sequence	Reference
*rpl*16 intron	F71	GCTATGCTTAGTTGTTGACTCGTTG	Jordan *et al.* (1996)
	R1661	CGTACCCATATTTTTCCACCACGA	Jordan *et al.* (1996)
	R1516	CCCTTCATTCTTCCTCTATGTTG	Kelchner & Clark (1997)
	R637	GGTTCGTTCCGCCATCC	Dickie & Wallace (1996)
	F543	TCAAGAAGCGATGGGAACGATGG	Butterworth *et al.* (2002)
*psb*A-*trn*H IGS	PsbAF	GTTATGCATG**G**ACGTAATGCTC	This paper
	TrnHR	CGCGCATGGTGGATTCACAAATC	Sang *et al.* (1997)

RPL16 INTRON. The *rpl*16 intron was amplified in 100 μL reactions which included 10 μL of 10X buffer, 5 μL of 25 mmol/L magnesium chloride solution, 8 μL of 25 mmol of an equimolar dNTP solution, 20 pmol of each primer (F71 and R1661), 0.5 μL of *Taq* polymerase, and 2 μL (<10ng) of DNA template. The following temperature cycling parameters gave sufficient amplification of the *rpl*16 intron: an initial melting at 95°C for 5 min followed by 24 cycles of the following protocol: 1) 95°C melt for 2 min; 2) 50°C annealing for 1 min; 3) ramp temperature increase of 15°C at 0.125°C per sec; 4) 65°C extension for 4 min. A final extension step at 65°C for 10 min completed the PCR amplification.

In 17 of the *Mammillaria* species sampled for this study, the *rpl*16 intron failed to amplify using all combinations of forward and reverse primers. To check for the presence of the intron, PCR amplifications were conducted for the entire *rpl*16 gene using primers RPL16F (Campagna and Downie, 1998) and R1661. Amplicons and subsequent sequences clearly demonstrated that in these species, the entire *rpl*16 intron has been deleted (Butterworth *et al.*, In Prep).

Table 5-3. Relative positions and lengths of binary encoded indels, and excluded regions (for the *rpl*16 intron) of unalignable sequence.

Sequence	Position	Length
*rpl*16 intron	29-32	4
	104-106	3
	203-212	10
	230-233	4
	317	1
	531-534	4
	617-639	23
	640-644	5
	645-685	41
	735-754	20
	727-838	112
	770-775	6
	875-880	6
	881-887	7
	888-892	5
	995-997	3
*rpl*16 intron – unalignable regions	588-615	28
	686-713	28
*psb*A-*trn*H IGS	28-41	14
	63-67	5
	68-74	7
	104-109	6
	110-115	6
	148-151	4
	225-312	88
	240-246	7
	294-297	4
	356-367	12

psbA-trnH Intergenic Spacer. The psbA-trnH IGS was amplified in 50 μL reactions which included 5 μL of 10X buffer, 2.5 μL of 25 mmol/L magnesium chloride solution, 4 μL of 25 mmol of an equimolar dNTP solution, 10 pmol of each primer (PSBAF and TRNHR), 0.25 μL of *Taq* polymerase, and 1 μL of unquantified DNA template. The following temperature cycling parameters gave sufficient amplification of the *psbA-trnH* IGS: an initial melting at 94°C for 2 min followed by 31 cycles of the following protocol: 1) 94°C melt for 1 min; 2) 50°C annealing for 1 min; 3) ramp temperature increase of 15°C at 0.125°C per sec; 4) 65°C extension for 2 min. A final extension step at 65°C for 10 min completed the PCR amplification.

Purification and Sequencing of PCR Products. PCR products were spun in a vacuum centrifuge to reduce their volumes to approximately 10 μL, run into a 1.5% TAE agarose gel. The amplicon bands were excised from the gel and cleaned using one of the following two methods: 1) Geneclean II kit (Bio 101) according to the manufacturer's instructions. Elution from the glassmilk pellet was achieved in 10 μL sterile distilled water followed by a second elution in 5 μL sterile distilled water; 2) QIAquick Gel Extraction kit (Quiagen) according to the manufacturer's instructions. Elution was in 30μL sterile distilled water followed by a second elution in 20 μL sterile distilled water, the purified product was further concentrated in a vacuum centrifuge to a final volume of approximately 10 μL. Purified PCR products from both protocols were quantified using agarose electrophoresis using a 1% gel in TAE buffer. 1 μL of concentrated, purified PCR product was run into the gel alongside a quantity standard that consisted of two lanes containing 10 μL and 5 μL respectively of ϕX174-*HAEIII* (Invitrogen) at a concentration of 25 μg/ml.

Sequence data were obtained in chain-termination reactions using the ABI Prism Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). Approximately 200 ng and 100 ng of purified PCR products were used for sequencing of the *rpl16* intron and *psbA-trnH* IGS respectively. Sequencing primers for the *rpl16* intron were F543, R637 and

R1516, and for the psbA-trnH IGS, the amplification primers were used for sequencing. Only partial sequences for the *rpl*16 intron were obtained with approximately 200 nucleotides from the beginning of the intron being omitted. Kelchner and Clark (1997) demonstrated very limited levels of sequence divergence in this region. For most of the sequencing reactions, 1:4 dilutions of the BigDye solution gave acceptable reads, however for some amplicons, dilutions of 1:1 BigDye solution were required to yield acceptable DNA sequences. Electrophoresis and automated sequence reading were undertaken at the Iowa State University Protein Facility using Perkin Elmer / Applied Biosystems automatic sequencing units (ABI Prism 377).

Phylogenetic Analysis

The alignment of sequences was carried out using AutoAssembler (Biosystems, 1995) and Se-Al (Rambaut, 1995). Sequencing was carried out manually following the principles of Kelchner and Clark (1997) for the alignment of non-coding DNA. Insertion/deletion events (indels) considered to be phylogenetically informative were coded in binary (presence/absence) following the treatment of Graham *et al.* (2000) and added to the end of the data matrix. There were two regions of doubtful homology in the *rpl*16 intron which totaled 56 nucleotides. These nucleotides were excluded from all subsequent analyses.

Parsimony Analyses. Parsimony analyses were undertaken using PAUP* 4.0b10 (Swofford, 2002). Both the *rpl*16 and *psbA-trnH IGS* were tested for phylogenetic signal by calculation of the g-statistic (Hillis and Huelsenbeck, 1992) for 10,000 random trees. All substitutions and indels were equally weighted. Due to the large number of taxa in the dataset, a number of heuristic search strategies were employed in order to maximize the likelihood of finding the most parsimonious tree(s) for the dataset. Heuristic searches were performed on separate and combined datasets. An initial heuristic search employed TBR branch swapping on a starting tree obtained by stepwise addition, saving multiple parsimonious trees with MAXTREES set to autoincrement as necessary. Further heuristic searches limited the

number of saved parsimonious trees to 1,000 (MAXTREES = 1000). Additional random-addition searches of 50 replications with each replicate limited to saving a maximum of 1000 parsimonious trees (NCHUCK=1000 CHUCKSCORE=1) were performed in an attempt to find islands of shorter trees. Bootstrap values for the combined datasets were calculated for 45 replicates each saving a maximum of 1000 trees. For the individual datasets, bootstrap values were calculated using the 'fast' option for 10,000 replicates. Decay estimates (Bremer, 1988) were calculated using the converse constraint method as implemented using Autodecay (Eriksson and Wikström, 1995).

CONGRUENCE TESTING. Both markers sampled for this study are located in the chloroplast and thus are inherited as a single unit such that phylogenies based upon these markers should yield congruent topologies. Although this has been demonstrated by numerous authors, including Cronn *et al.* (2002) who clearly showed congruence for four chloroplast markers in cotton and by Nyffeler (2002) for two chloroplast markers in the Cactaceae, we felt that congruence testing should still be a fundamental part of analysis when dealing with multiple datasets. For this reason, congruence between the *rpl16* intron and *PsbA-trnH IGS* datasets was tested using two methods. The Incongruence Length Difference (ILD) test (Farris *et al.*, 1995) as implemented the Partition Homogeneity test in PAUP* was used to assess congruence between the *psbA-trnH IGS* and the *rpl16* intron datasets for 25 replicates each saving a maximum of 1,000 most parsimonious trees per replicate.

BAYESIAN ANALYSES. Phylogenetic reconstruction of discrete data (such as molecular sequences) using a Bayesian approach has become increasingly more popular as an alternative to Maximum Likelihood models, mainly because Bayesian methods are much less computationally intensive. Given the large number of taxa in our dataset, we opted for Bayesian rather than Maximum Likelihood analyses. Five independent Bayesian analyses were performed on the combined dataset using the software 'MrBayes' (Huelsenbeck and Ronquist, 2001, 2002). Each analysis was initiated from a random tree and run in a Markov chain for 1×10^6 cycles

Table 5-4. Summary of sequences of the *rpl16* intron, *psbA-trnH IGS* and combined datasets. [1] The is the number of sites after the exclusion of unalignable regions. [2] The number of informative indels for the *rpl16* intron includes the presence/absence of the entire intron.

Sequence Characteristics	*rpl16* intron	*psbA-trnH* IGS	Combined data
Length of aligned matrix (sites)	1036[1]	367	1403
Number of informative gaps	16[2]	9	25
Number of informative sites (% of total sites)	163 (16%)	80 (22%)	243 (17%)

with tree sampling every 100th cycle in the chain. Four chains were run simultaneously for each analysis and the initial 500 sampled trees (5%) were discarded as 'burn-in'. Settings for the model (LSET) used for analysis were - NUCMODEL=4by4 (the standard model of DNA substitution); NST=2 (HKY85 model); RATES=gamma (gamma-distribution of rates across sites); NGAMMACAT=4 (number of gamma rate categories); OMEGAVAR=equal (sets the nonsynonymous/synonymous rate ratio to equal); COVARION=no (do not use a covarian-like model of substitution); CODING=all (all site patterns were potentially sampled); PARSMODEL=no (do not use the parsimony model).

Results

Sequence length of the *rpl*16 intron varied considerably among those taxa in which it was present. The shortest sequences of the *rpl*16 intron were observed in *M. blossfeldiana* and *M. goodridgei* (589 bp) and *M. mammillaris* (615 bp), and the longest sequences were observed in *Escobaria hesteri* (964 bp) and *M. wrightii* (949 bp). Sequence length variation in the *psbA-trnH* IGS was much more uniform than in the *rpl*16 intron, and ranged from 206 bp in *Mammillaria candida* to 307 bp in *Stenocactus lloydii.* Length characteristics of the aligned sequences are summarized in Table 5-3. The aligned sequence length of the full dataset (including binary-coded indels) totaled 1428bp. Including the binary-encoded indels, the dataset contained 268 parsimony informative sites. There appears to be considerable phylogenetic

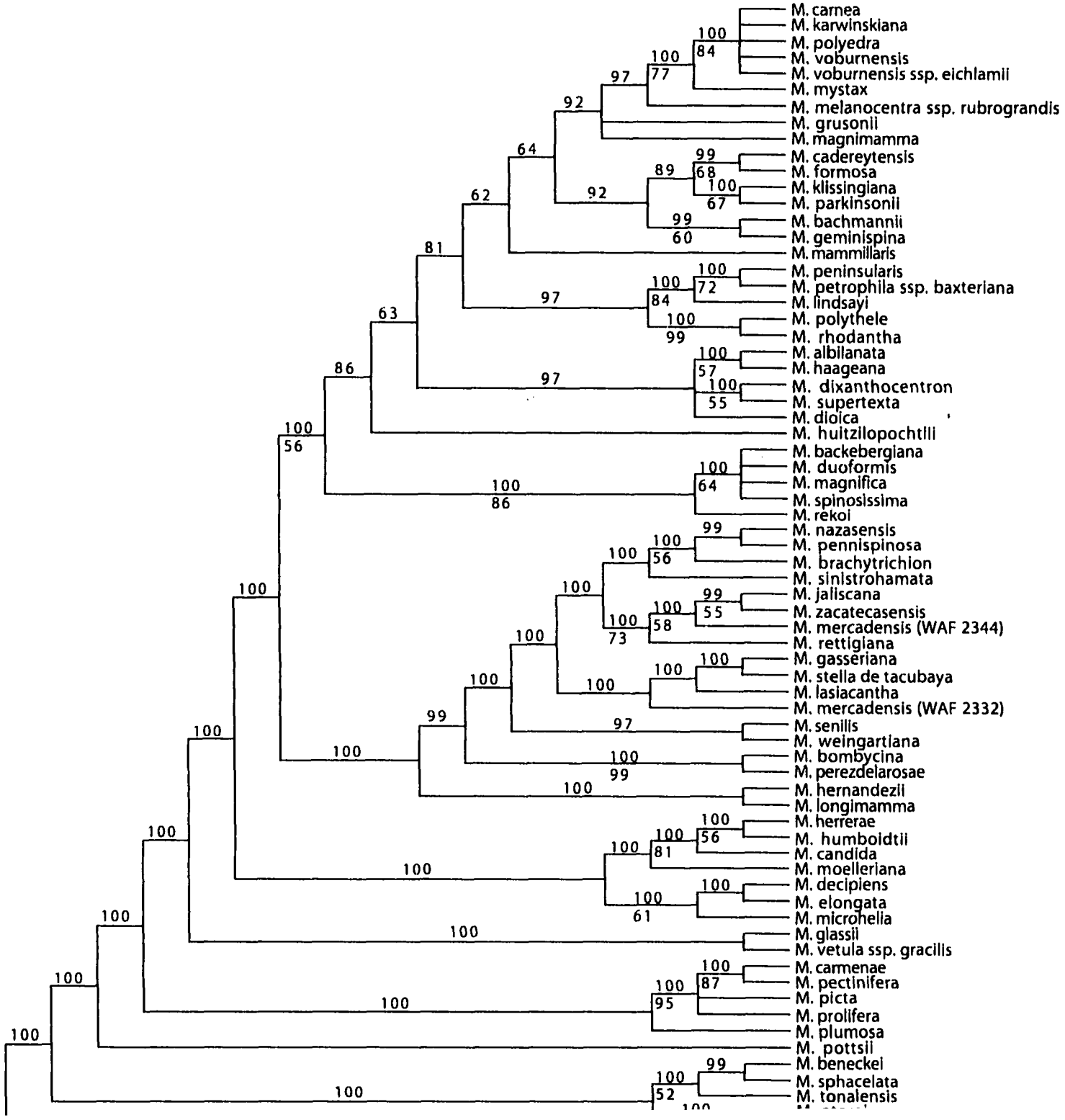
signal in the *psbA-trnH* IGS, *rpl16* intron and combined data matrices with g-statistics of –0.399, –0.300, and –0.355 respectively. All of these fall within the 95% and 99% confidence limits for 25 taxa and 500 characters (Hillis and Huelsenbeck, 1992).

Parsimony Analyses

The results of the heuristic searches are summarized in Table 5-4. Heuristic searches on the individual datasets did not find the most parsimonious trees when MAXTREES was set to autoincrement. The trees found and saved by these searches exceeded 150,000 in number and caused PAUP to run out of memory on a Macintosh G4 computer with 990Mb of memory. For this reason, subsequent heuristic searches were limited to saving a maximum of 1,000 trees (MAXTREES=1000), and under this option shorter trees were found (see Table 5-4). Random addition searches failed to find islands of shorter trees. Majority-rule consensus trees for the *rpl16* intron and *psbA-trnH* IGS are shown in Figures 5-2 and 5-3 respectively.

Table 5-5. Summary of parsimony analyses of the *rpl16* intron, *psbA-trnH IGS* and combined datasets. Data reported is for strict / majority-rule consensus trees. [1] Taxa lacking the *rpl*16 intron pruned from the dataset. [2] The number of resolved clades is for majority rule trees recovered from heuristic searches. [3] Resolution Index is the percentage of clades recovered versus the maximum number of possible clades in a bifurcating tree that has n taxa in the ingroup (for datasets with all taxa included, the number of bifurcating clades is n-1 = 124 clades, and for those datasets with taxa lacking the *rpl16* intron excluded, n-1 = 107).

Analysis data	*rpl16* intron[1]	*psbA-trnH IGS*	Combined data
Tree Length	663/626	282/276	943/918
Consistency Index	0.6078/0.6438	0.5887/0.6014	0.6045/0.6209
Consistency Index (excl. uninformative characters)	0.4821/0.5204	0.5105/0.5238	0.4939/0.5112
Homoplasy Index	0.3922/0.3562	0.4113/0.3986	0.3955/0.3791
Homoplasy Index (excl. uninformative characters)	0.5179/0.4796	0.4895/0.4762	0.5061/0.4888
Retention Index	0.7994/0.8279	0.8020/0.8123	0.8035/0.8166
Number of resolved clades [2]	62/94	57/90	80/106
Resolution Index[3]	0.58/0.88	0.46/0.72	0.65/0.85


M. carnea
M. karwinskiana
M. polyedra
M. voburnensis
M. voburnensis ssp. eichlamii
M. mystax
M. melanocentra ssp. rubrograndis
M. grusonii
M. magnimamma
M. cadereytensis
M. formosa
M. klissingiana
M. parkinsonii
M. bachmannii
M. geminispina
M. mammillaris
M. peninsularis
M. petrophila ssp. baxteriana
M. lindsayi
M. polythele
M. rhodantha
M. albilanata
M. haageana
M. dixanthocentron
M. supertexta
M. dioica
M. hultzilopochtlii
M. backebergiana
M. duoformis
M. magnifica
M. spinosissima
M. rekoi
M. nazasensis
M. pennispinosa
M. brachytrichion
M. sinistrohamata
M. jaliscana
M. zacatecasensis
M. mercadensis (WAF 2344)
M. rettigiana
M. gasseriana
M. stella de tacubaya
M. lasiacantha
M. mercadensis (WAF 2332)
M. senilis
M. weingartiana
M. bombycina
M. perezdelarosae
M. hernandezii
M. longimamma
M. herrerae
M. humboldtii
M. candida
M. moelleriana
M. decipiens
M. elongata
M. microhelia
M. glassii
M. vetula ssp. gracilis
M. carmenae
M. pectinifera
M. picta
M. prolifera
M. plumosa
M. pottsii
M. beneckei
M. sphacelata
M. tonalensis

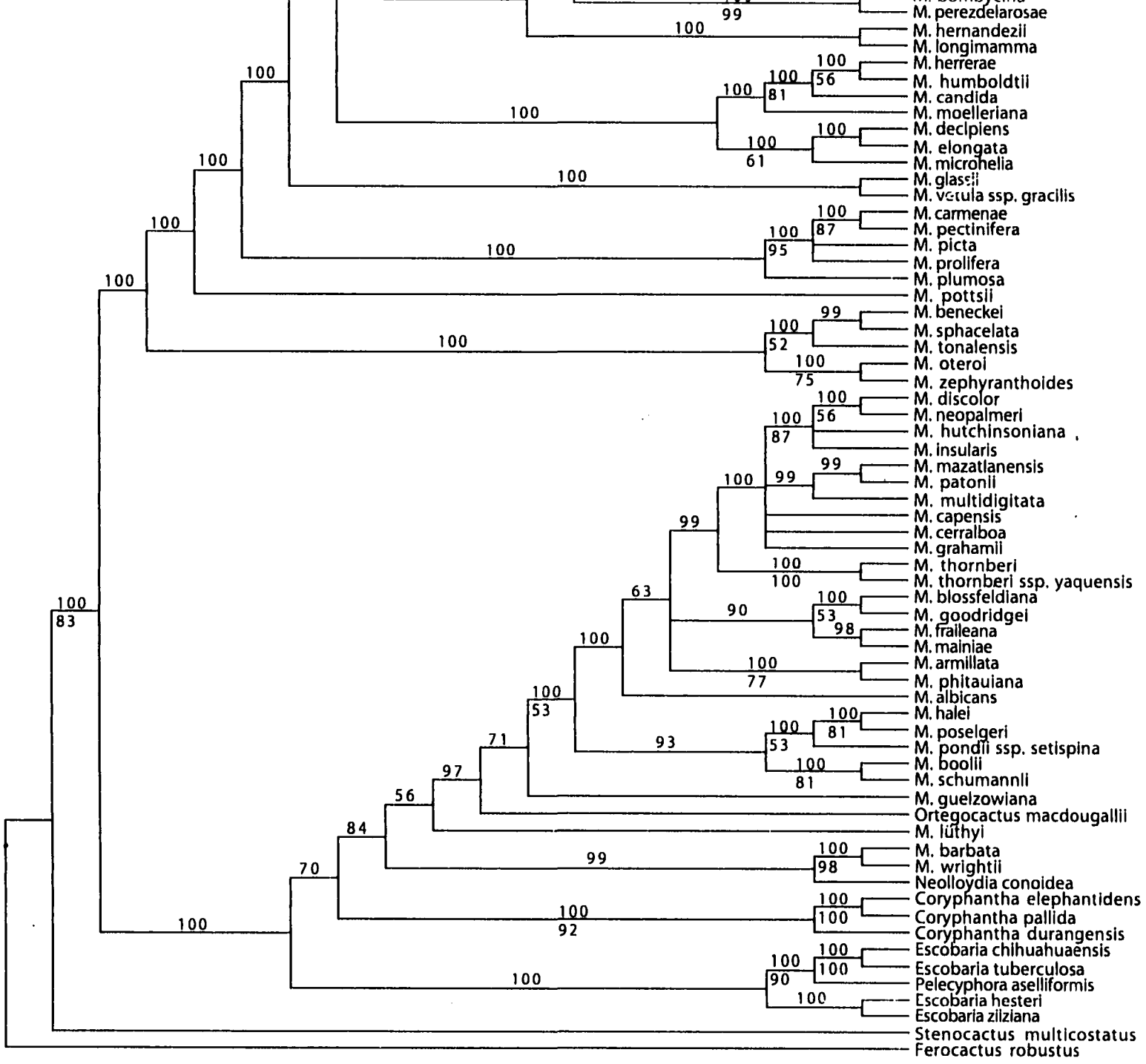


Figure 5-2. Majority-rule consensus of 1,000 most parsimonious trees for *rpl*16 intron sequence data. Percentage support is shown above the branch. Bootstrap values greater than 50% are shown below the branches. WAF collection numbers are shown for multiple accessions of *M. mercadensis*.

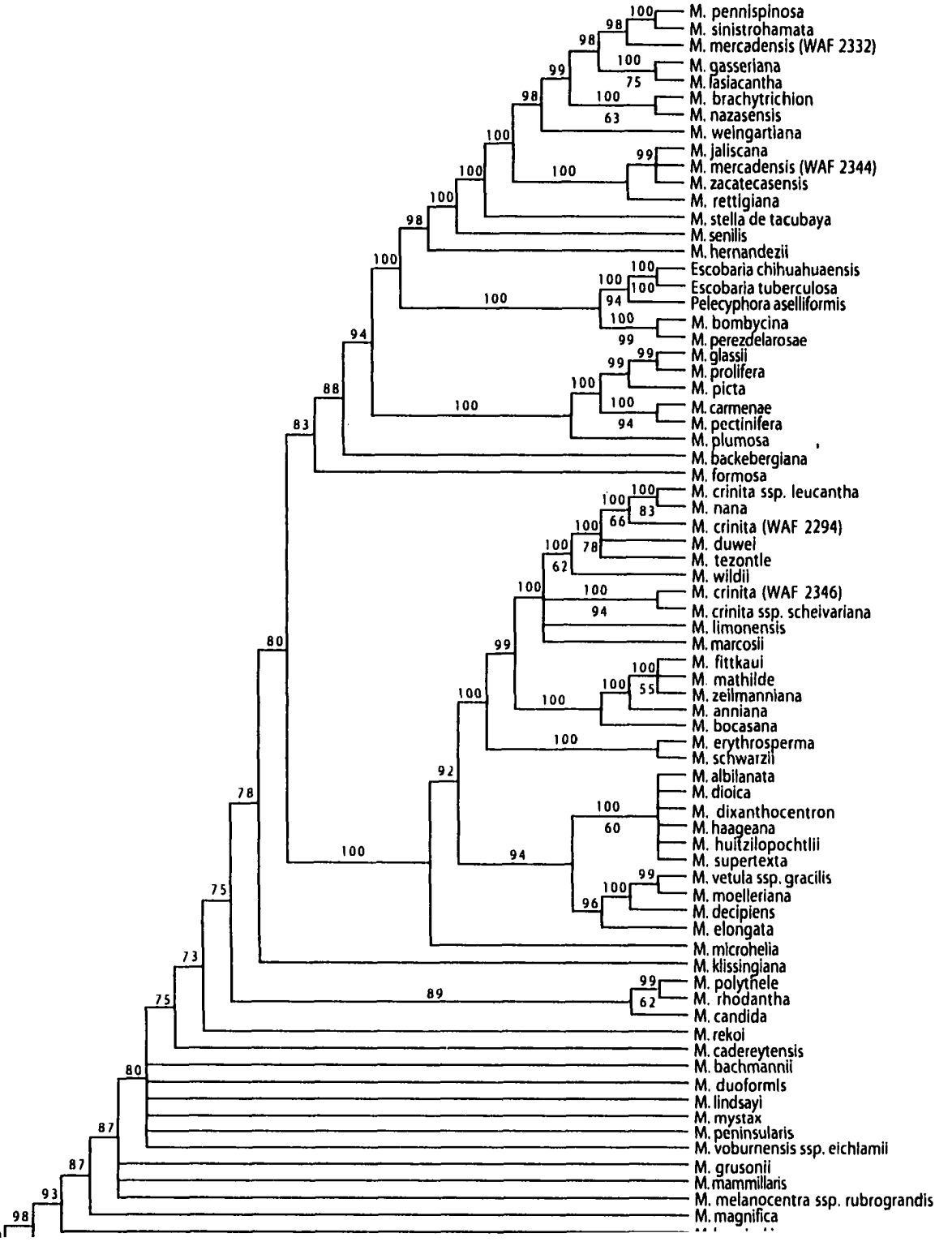
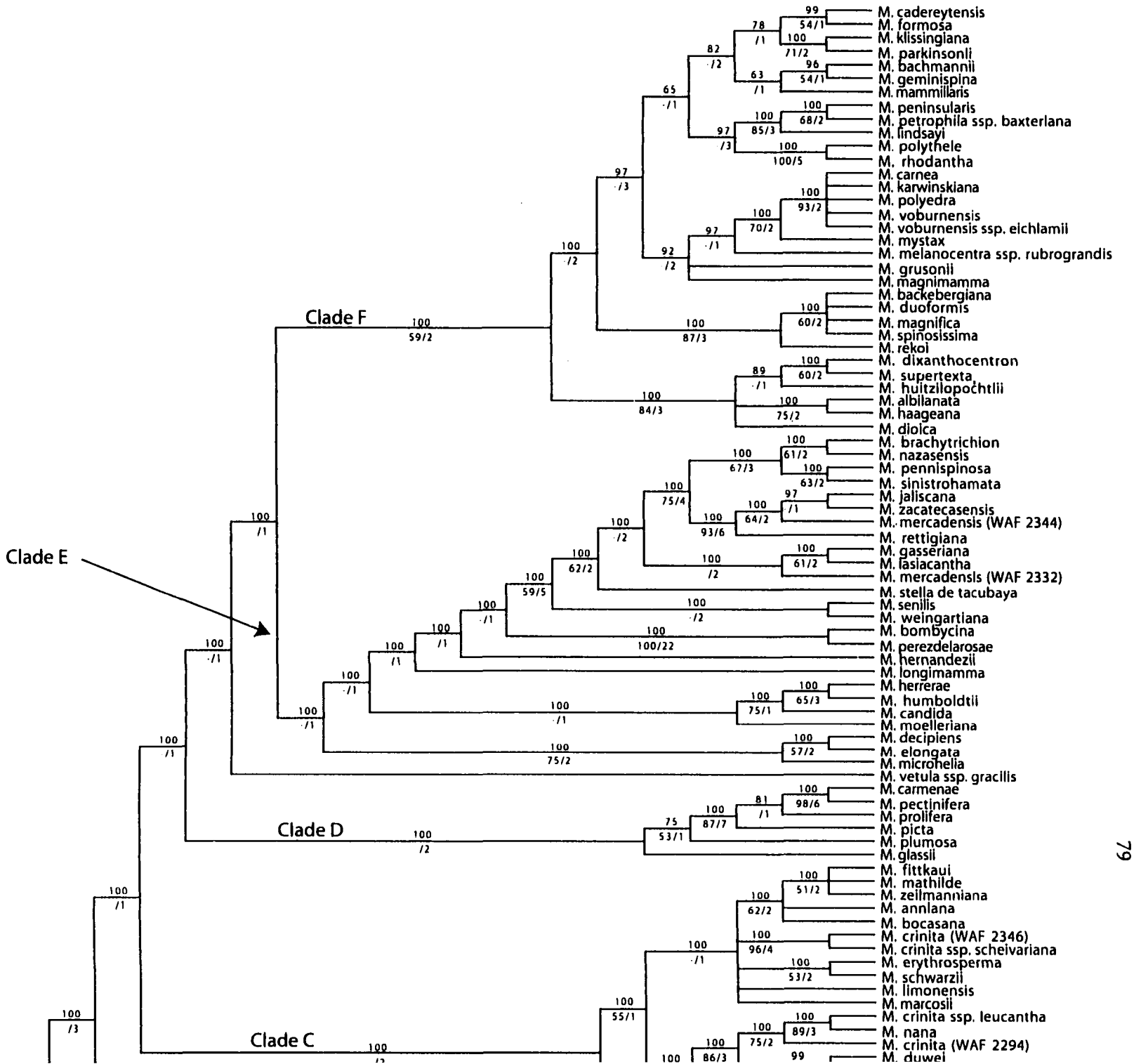

M. pennispinosa
M. sinistrohamata
M. mercadensis (WAF 2332)
M. gasseriana
M. lasiacantha
M. brachytrichion
M. nazasensis
M. weingartiana
M. jaliscana
M. mercadensis (WAF 2344)
M. zacatecasensis
M. rettigiana
M. stella de tacubaya
M. senilis
M. hernandezii
Escobaria chihuahuaensis
Escobaria tuberculosa
Pelecyphora aselliformis
M. bombycina
M. perezdelarosae
M. glassii
M. prolifera
M. picta
M. carmenae
M. pectinifera
M. plumosa
M. backebergiana
M. formosa
M. crinita ssp. leucantha
M. nana
M. crinita (WAF 2294)
M. duwei
M. tezontle
M. wildii
M. crinita (WAF 2346)
M. crinita ssp. scheivariana
M. limonensis
M. marcosii
M. fittkaui
M. mathilde
M. zeilmanniana
M. anniana
M. bocasana
M. erythrosperma
M. schwarzii
M. albilanata
M. dioica
M. dixanthocentron
M. haageana
M. huitzilopochtlii
M. supertexta
M. vetula ssp. gracilis
M. moelleriana
M. decipiens
M. elongata
M. microhelia
M. klissingiana
M. polythele
M. rhodantha
M. candida
M. rekoi
M. cadereytensis
M. bachmannii
M. duoformis
M. lindsayi
M. mystax
M. peninsularis
M. voburnensis ssp. eichlamii
M. grusonii
M. mammillaris
M. melanocentra ssp. rubrograndis
M. magnifica

Figure 5-3. Majority-rule consensus of 1,000 most parsimonious trees for *psbA-trnH* IGS sequence data. Percentage support is shown above the branch. Bootstrap values greater than 50% are shown below the branches. WAF collection numbers are shown for multiple accessions of *M. mercadensis* and *M. crinita*.

Making assessments regarding the utility of the different datasets for producing robust phylogenies is not simple. Using standard measures, it would appear that the *rpl16* intron with 163 informative sites and 16 scored indels should produce a better resolved phylogeny than the *psbA-trnH IGS* which only has 80 informative sites and 9 scored indels. Indeed with 22% of its sites being parsimony informative, we could reason that the *psbA-trnH IGS* should include more multiple hits than the *rpl16* intron which only has 16% informative sites. A visual comparison of trees (strict and majority-rule consensuses) produced may be a suitable indicator of the resolving powers of particular markers. However, in order to produce a numeric comparison of 'resolving power' of the two datasets used in the this study, we opted to create a 'Resolution Index' for individual markers and combined dataset for both the strict and majority rule consensus trees. A fully-resolved, bifurcating rooted-tree contains n-1 clades, where n is the number of taxa in the ingroup. The 'Resolution Index' is simply the proportion of clades recovered in parsimony analysis to the maximum number of possible clades (from the above equation). This index gives a very clear and easily interpretable indication of how sufficiently different datasets produce fully-resolved trees, either as a comparison between markers for a single set of taxa (as in this study) or between different taxa or taxonomic ranks for a single marker.

The *rpl16* and *psbA-trnH* IGS datasets show significantly high degrees of congruence. The ILD tests gave a p-value of 0.8 which suggests that the null hypothesis (tree lengths from random partitions being statistically similar to those from the original partitions) should not be rejected (see Johnson and Soltis, 1998). This result indicates that the datasets can be combined. The majority-rule consensus tree from the combined *rpl16* intron and *psbA-trnH* IGS is shown in Figure 5-4.

The majority-rule consensus (Figure 5-4) reveals a major basal dichotomy that distinguishes two major groups of *Mammillaria*. 28 of the sampled species of *Mammillaria* form a clade (Clade A) that is sister to sampled species of *Coryphantha*, *Escobaria* and *Pelecyphora*.


Clade E
Clade F
Clade D
Clade C
M. cadereytensis
M. formosa
M. klissingiana
M. parkinsonii
M. bachmannii
M. geminispina
M. mammillaris
M. peninsularis
M. petrophila ssp. baxterlana
M. lindsayi
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M. duoformis
M. magnifica
M. spinosissima
M. rekoi
M. dixanthocentron
M. supertexta
M. hultzilopochtlii
M. albilanata
M. haageana
M. dioica
M. brachytrichion
M. nazasensis
M. pennispinosa
M. sinistrohamata
M. jaliscana
M. zacatecasensis
M. mercadensis (WAF 2344)
M. rettigiana
M. gasseriana
M. lasiacantha
M. mercadensis (WAF 2332)
M. stella de tacubaya
M. senilis
M. weingartiana
M. bombycina
M. perezdelarosae
M. hernandezii
M. longimamma
M. herrerae
M. humboldtii
M. candida
M. moelleriana
M. decipiens
M. elongata
M. microhelia
M. vetula ssp. gracilis
M. carmenae
M. pectinifera
M. prolifera
M. picta
M. plumosa
M. glassii
M. fittkaui
M. mathildae
M. zeilmanniana
M. annlana
M. bocasana
M. crinita (WAF 2346)
M. crinita ssp. scheivariana
M. erythrosperma
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M. nana
M. crinita (WAF 2294)
M. duwei

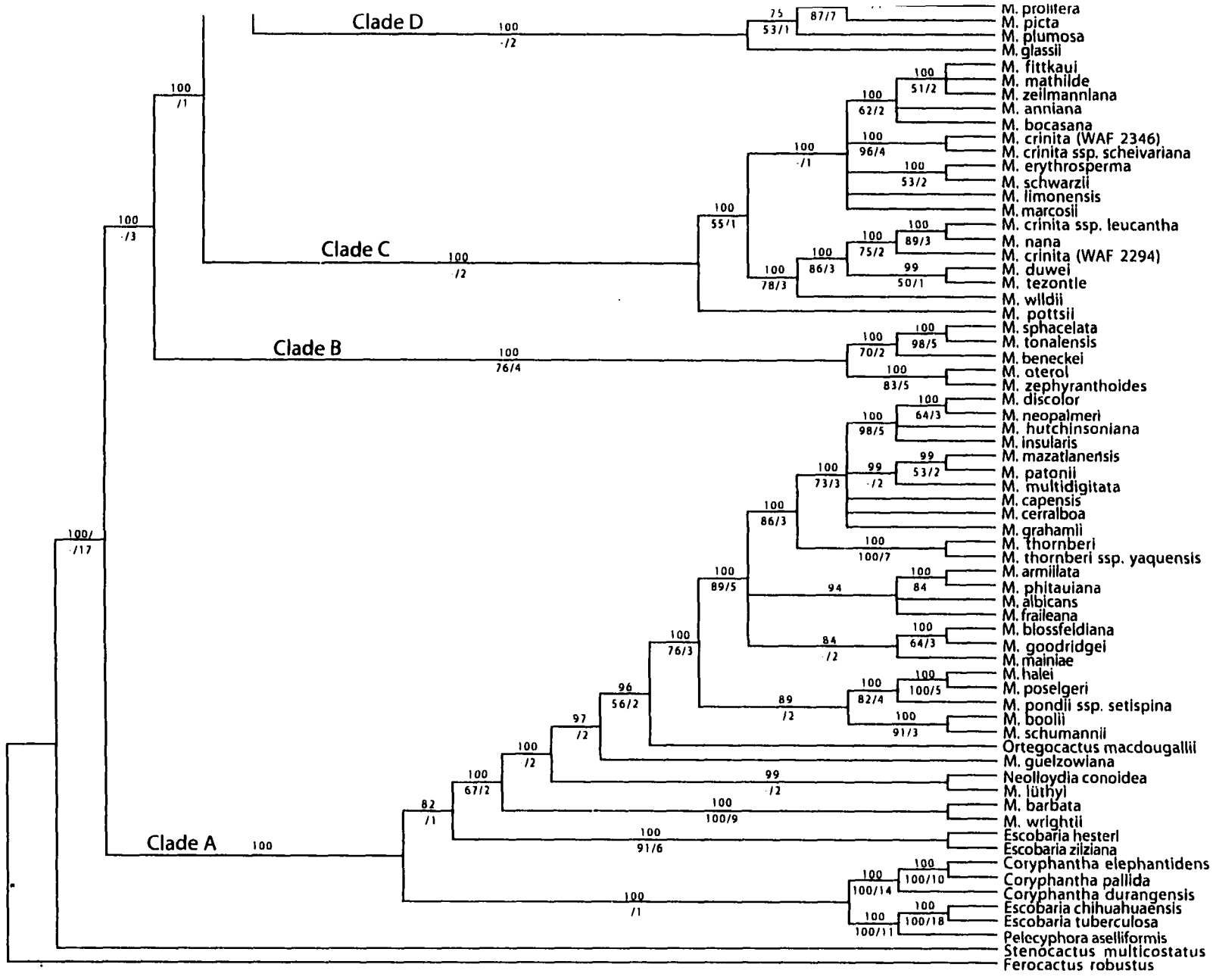
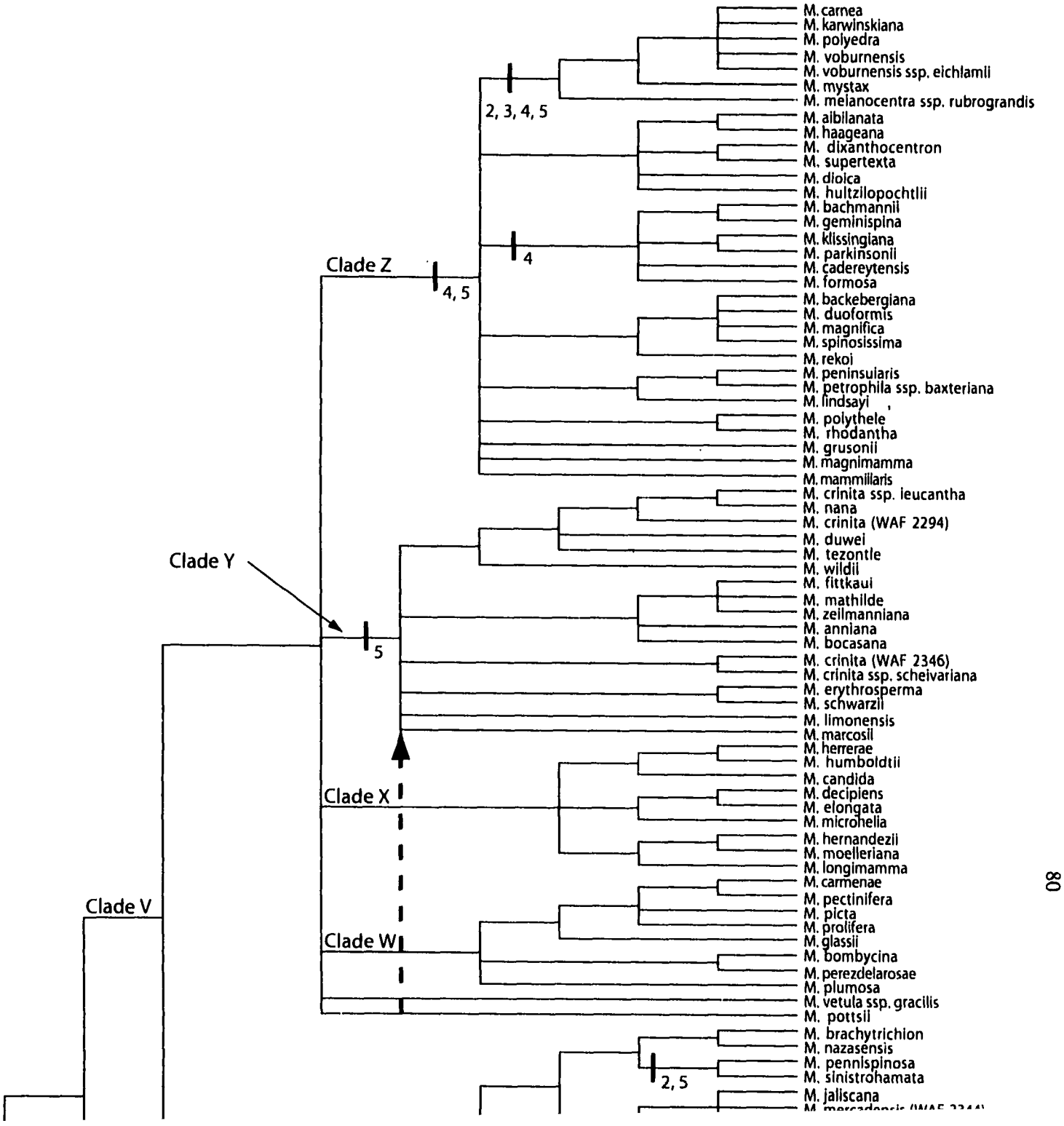


Figure 5-4. Majority-rule consensus of 1,000 most parsimonious trees for combined *rpl*16 intron and *psb*A-*trn*H IGS sequence data. Percentage support for majority-rule is shown above the branch. Bootstrap values greater than 50% are shown below the branches. Decay values are shown below the branches following the bootstrap values. WAF collection numbers are shown for multiple accessions of *M. mercadensis* and *M. crinita*.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M. karwinskiana
M. polyedra
M. voburnensis
M. voburnensis ssp. eichlamii
M. mystax
M. melanocentra ssp. rubrograndis
2, 3, 4, 5
M. aibilanata
M. haageana
M. dixanthocentron
M. supertexta
M. dioica
M. hultzilopochtlii
M. bachmannii
M. geminispina
4
M. klissingiana
M. parkinsonii
M. cadereytensis
M. formosa
Clade Z
4, 5
M. backebergiana
M. duoformis
M. magnifica
M. spinosissima
M. rekoi
M. peninsularis
M. petrophila ssp. baxteriana
M. lindsayi
M. polythele
M. rhodantha
M. grusonii
M. magnimamma
M. mammillaris
M. crinita ssp. leucantha
M. nana
M. crinita (WAF 2294)
M. duwei
M. tezontle
M. wildii
Clade Y
M. fittkaui
M. mathilde
M. zeilmanniana
M. anniana
M. bocasana
5
M. crinita (WAF 2346)
M. crinita ssp. scheivariana
M. erythrosperma
M. schwarzii
M. limonensis
M. marcosii
M. herrerae
M. humboldtii
M. candida
Clade X
M. decipiens
M. elongata
M. microhelia
M. hernandezii
M. moelleriana
M. longimamma
M. carmenae
Clade V
M. pectinifera
M. picta
M. prolifera
Clade W
M. glassii
M. bombycina
M. perezdelarosae
M. plumosa
M. vetula ssp. gracilis
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2, 5
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M. jaliscana

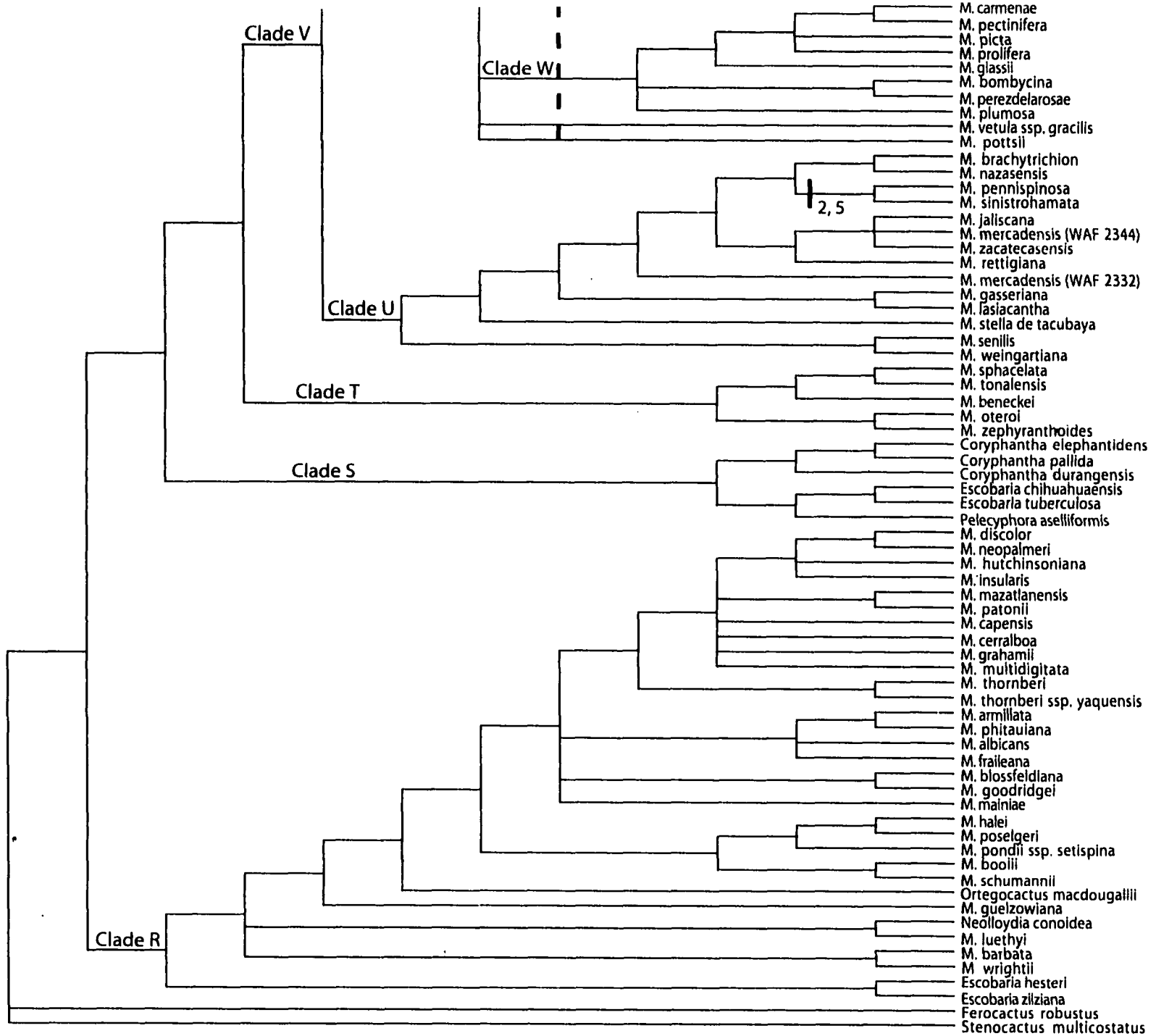

Figure 5-5. Majority-rule consensus trees from Bayesian analysis 1. Numbered branches indicate the collapsed clades in Bayesian analyses 2 through 5. The dashed arrow shows the placement of Mammillaria pottsii in majority-rule trees produced by Bayesian searches 2 through 5. WAF collection numbers are shown for multiple accessions of *M. mercadensis* and *M. crinita*.

Within 'Clade A', there are two non-*Mammillaria* taxa – *Neolloydia conoidea* and *Ortegocactus macdougallii*. The second group of the major basal dichotomy contains the remaining *Mammillaria* taxa sampled in this study. Within this group of mammillarias there are a number of resolved clades: 1. 'Clade B' consists of five species – *M. beneckei, M. oteroi, M sphacelata, M. tonalensis* and *M. zephyranthoides* (bootstrap 76, decay 4); 2. 'Clade C' – members of series *Stylothelae* (*sensu* Hunt) including *M. pottsii* (bootstrap < 50%, decay 2); 3. 'Clade D' – *M. carmenae, M. glassii, M. pectinifera, M. picta, M. plumosa* and *M. prolifera* (bootstrap < 50%, decay 2); 4. *M. vetula* ssp. *gracilis* which forms the sister group to a large clade that forms a dichotomy of the two remaining clades; 5. 'Clade E' – remaining members of series *Stylothelae* (*sensu* Hunt) and *M. hernandezii, M. longimamma, M. herrerae, M. humboldtii, M. candida, M. decipiens, M. elongata* and *M. microhelia* (bootstrap < 50%, decay 1); 6. 'Clade F' – a large clade containing the remaining 32 sampled taxa of *Mammillaria* (bootstrap 59%, decay 2).

Bayesian Analyses

The five individual Bayesian analyses produced majority-rule trees that are topologically congruent. The number of clades resolved in the five analyses had between 79 resolved clades in analysis 5 (Resolution Index = 0.64) and 84 resolved clades in analysis 1 (Resolution Index = 0.68). Analyses 2 and 3 resolved 82 clades and analysis 4 resolved 81 clades. The majority-rule tree from the first Bayesian analysis is shown in Figure 5-5. Between this tree and the trees from the other four analyses, there are a total of six differences (shown in Figure 5-5), all involving the collapse of individual clades rather than drastic topological rearrangements.

The majority-rule consensus tree from the first Bayesian analysis reveals a major basal dichotomy within the ingroup taxa. 'Clade R' includes *Escobaria hesteri* and *E. zilziana*, which form a sister-clade to a clade containing 28 of the *Mammillaria* taxa studied, *Neolloydia*

conoidea and *Ortegocactus macdougallii*. The second clade of the major basal dichotomy also resolves a number of distinct clades: 1. 'Clade S' – the sampled members of genus *Coryphantha* reside in this clade along with *Escobaria chihuahuaensis* and *E. tuberculosa*; 2. 'Clade T' – *M. beneckei, M. oteroi, M. sphacelata, M. tonalensis* and *M. zephyranthoides*; 3. 'Clade U' – members of series *Stylothelae* (*sensu* Hunt) plus *M. senilis*; 4. 'Clade V' – sister group to 'Clade U', this clade contains the remaining 68 taxa of *Mammillaria* which are further divided among four distinct clades that form a polychotomy with *M. vetula* ssp. *gracilis* and *M. pottsii*; 5. 'Clade W' – *M. carmenae, M. pectinifera, M. picta, M. prolifera, M. glassii, M. bombycina* and *M. perezdelarosae*; 6. 'Clade X' – *M. herrerae, M. humboldtii, M. candida, M. decipiens, M. elongata, M. microhelia, M. hernandezii, M. moelleriana* and *M. longimamma*; 7. 'Clade Y' – containing the remaining species of series *Stylothelae* (*sensu* Hunt); 8. 'Clade Z' – large clade containing 32 of the sampled *Mammillaria* taxa.

A Comparison of Parsimony and Bayesian Trees

The majority-rule consensus tree from the first Bayesian analysis (Figure 5-5) resolved fewer clades (Resolution Index = 0.68) than the majority-rule consensus tree from the parsimony analysis (Figure 5-4) which had a Resolution Index of 0.85. In spite of the differences in Resolution Index, both methods of phylogenetic reconstruction produced trees that were not dramatically dissimilar. Figure 5-6 compares parsimony and Bayesian trees in which clades A – F (in the parsimony tree) and R – Z (in the Bayesian tree) have been reduced to terminal taxa. Both methods of phylogeny reconstruction show a fairly nested arrangement of clades within the ingroup: 1. 'Clade A' in the parsimony tree is equivalent to 'Clade R' from the Bayesian analysis with the exception that members of 'Clade S' are included in 'Clade A'; 2. 'Clade B' and 'Clade T' are identical in their membership and placement of the clade as sister to the remaining sampled taxa of *Mammillaria*; 3. 'Clade C' of the parsimony analysis is equivalent to 'Clade Y' of the Bayesian analysis with the exception that *M. pottsii*

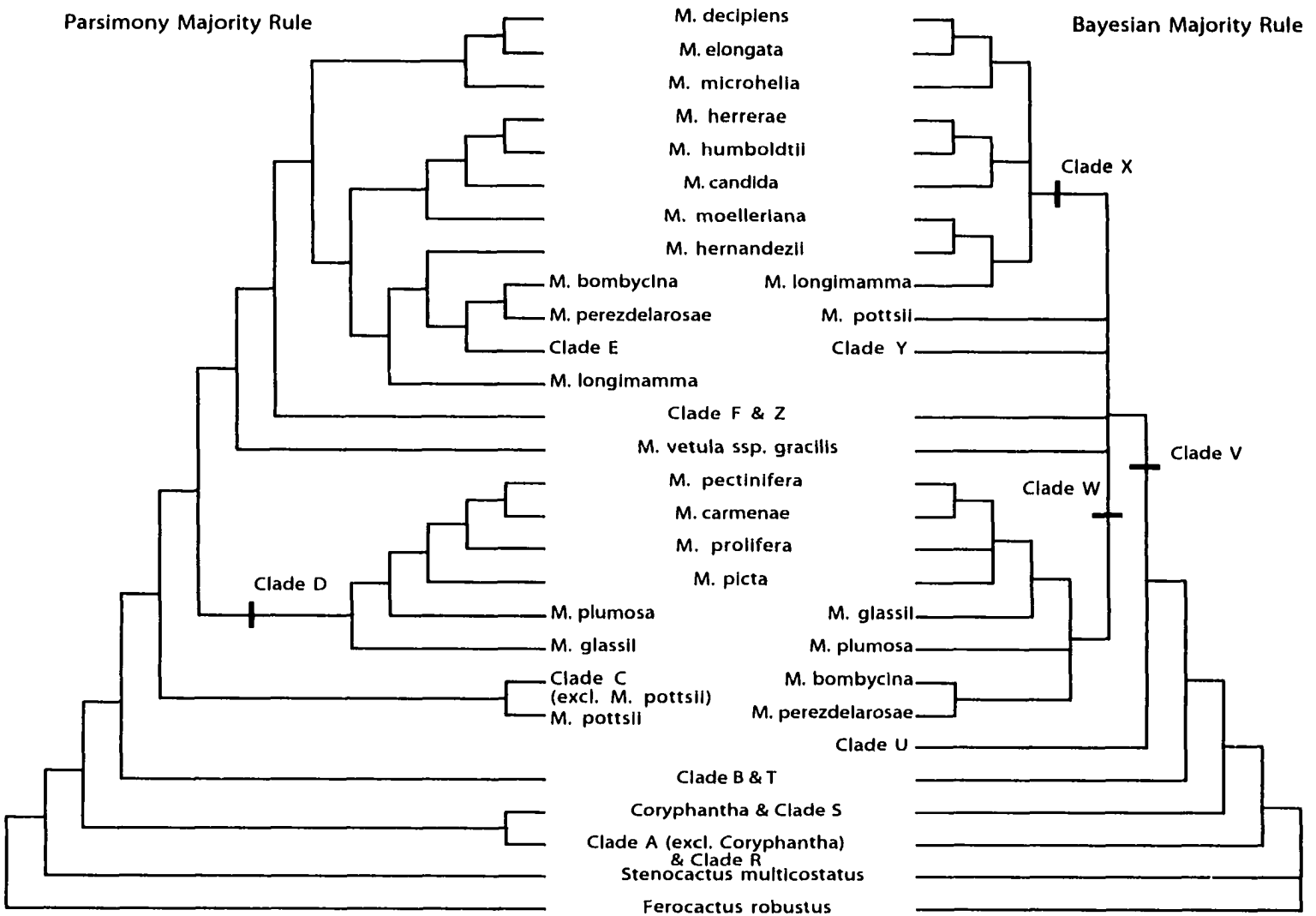

Figure 5-6. Comparison of the majority-rule consensus trees from the parsimony analysis and Bayesian analysis 1. Major clades described in the results section have been collapsed where there is direct congruence between both trees. Clades A through F are major clades recovered in the parsimony analysis and clades R through Z are major clades from Bayesian analysis 1.

is excluded from 'Clade Y' and the position of the clades are different between both analyses – in the Bayesian tree, 'Clade Y' is placed within 'Clade V'; 4. Clades 'D' and 'W' share similar a membership to each other, the exception being the placement of *M. bombycina* and *M. perezdelarosae* which are included in 'Clade W' but not 'Clade D'; 5. In the parsimony reduced clades majority-rule tree, the remaining sampled taxa of *Mammillaria* form a nested series of clades that is fully resolved. This does not occur in the Bayesian majority-rule tree which resolves a polychotomy within 'Clade V'; 6. 'Clade X' in the Bayesian tree is not supported in the parsimony analyses, although subclade groupings are fairly congruent between both analyses. The major differences between the reduced clades majority-rule consensus trees from the parsimony and Bayesian analyses lie in the interchange of position of clades 'E' / 'U' with clades 'C' / 'Y', and the position of *M. bombycina, M. longimamma, M. perezdelarosae* and *M. pottsii.*

Discussion

Phylogenetic Relationships in *Mammillaria*

Based upon the phylogeny produced from the parsimony analyses (Figure 5-4), a number of conclusions can be drawn regarding phylogenetic relationships in *Mammillaria.*

Monophyly of *Mammillaria*. The ingroup for this study includes members of the 'Mammilloid' Clade defined by Butterworth, Cota-Sanchez and Wallace (2002). Within this clade, the placement of *Neolloydia conoidea* and *Ortegocactus macdougallii* rendered the genus *Mammillaria* paraphyletic. The increased sampling of *Mammillaria* species in this study affirms that as currently circumscribed the genus is not monophyletic. Transferring *Neolloydia conoidea* and *Ortegocactus macdougallii* into *Mammillaria* would not solve the paraphyly of *Mammillaria* due to the placement of *Escobaria, Coryphantha* and *Pelecyphora* species sampled. Further sampling of taxa from the genera *Coryphantha* and *Escobaria* is required to more clearly elucidate their relationships to *Mammillaria.*

CLADE A. With the exclusion of the non-*Mammillaria* taxa, Clade 'A' corresponds favorably with Hunt's (1981) circumscription of series *Ancistracanthae* and Lüthy's (1995; 2001) subgenus *Cochemiea*. Members of series *Ancistracanthae* are often slenderly cylindric and densely clustering with stout, firm tubercles. Central spines of the spine-bearing areoles are typically hooked, although some species have straight spines. Flowers of series *Ancistracanthae* tend to be large (relative to other species in *Mammillaria*), funnelform and color ranges from purplish-pink to creamy-yellow to white. Their distribution is predominantly in N.W. Mexico and S.W. United States. However, embedded within series *Ancistracanthae* (*sensu* Hunt) is subgenus *Cochemiea* (*sensu* Hunt), whose species (represented in the study by *M. poselgeri*, *M. halei* and *M. pondii* ssp. *setispina*) are very distinct in *Mammillaria* for their elongated cylindrical stems that may be either upright or prostrate, and flowers that are unique in *Mammillaria* for their narrowly tubular shape with bilateral symmetry and hummingbird pollination. A number of authors (Anderson, 2001; Berger, 1926, 1929; Britton and Rose, 1923; Buxbaum, 1951c, 1958) recognized *Cochemiea* at the level of genus. The phylogeny presented in this study suggests that in spite of unique gross morphology, the recognition of *Cochemiea* at a rank equal to or higher than series, would render paraphyletic Hunt's circumscription of series *Ancistracanthae*. Other non-*Ancistracanthae* species of *Mammillaria* included within Clade 'A' include *M. discolor* and *M. luethyi*. With morphology and distribution somewhat different from the typical *Ancistracanthae*, *M. discolor* sits uncomfortably in this group to the extent that it has been placed in the entirely unlike series *Heterochlorae* by both Hunt and Lüthy. *Mammillaria luethyi* is probably one of the most recognizable species of the genus, in having minute spines that branch repeatedly near their apex. Originally discovered by Norman Boke in Coahuila, Mexico in 1952 as a cultivated specimen, the species went undescribed and all cultivated material was eventually lost. George Hinton and Jonas Lüthy subsequently rediscovered the plant in habitat in 1996 and it was described by George

Hinton. Hunt (1997) places *M. luethyi* in series *Lasiacanthae* with other species that possess mainly undifferentiated numerous diminutive spines.

Clade 'A' as circumscribed in Figure 5-4 includes sampled members of the gerera *Coryphantha*, *Escobaria* and *Pelecyphora*, which form sister lineages to sampled taxa of Hunt's and Lüthy's series *Ancistracanthae* and subgenus *Cochemiea* respectively thus clearly demonstrating paraphyly within *Mammillaria*. However, within the core group of series *Ancistracanthae sensu* Hunt and subgenus *Cochemiea sensu* Lüthy, our phylogeny places *Ortegocactus macdougallii* and *Neolloydia conoidea*. Discovered by MacDougall in the early 1950's and described by Alexander in 1961, *Ortegocactus macdougallii* has been contentious in its placement in relation to other members of tribe Cacteae. Bravo-Hollis and Sánchez-Mejorada (1991) sank this genus into *Neobesseya*, members of which are now commonly accepted as species of *Escobaria* (Anderson, 2001; Barthlott and Hunt, 1993; Hunt, 1992, 1999). Hunt and Taylor (1986; 1990) suggested that *Ortegocactus* may be referable to the genus *Mammillaria*, although an official transfer to *Mammillaria* was not made. Barthlott and Hunt (1993) also commented on the similarities of *Ortegocactus* and *Mammillaria* going so far as to suggest that *Ortegocactus* is reminiscent of *Mammillaria schumannii*. Butterworth *et al.* (2002) also suggested that *Ortegocactus* shared a greater affinity with members of *Mammillaria* than with *Escobaria* or *Coryphantha*. The data presented in this paper does indeed show that *Ortegocactus macdougallii* is embedded within members of *Mammillaria*, its closest *Mammillaria* relatives including *M. schumannii*. However, at present the transfer of *Ortegocactus* to *Mammillaria* would be inappropriate due to the polyphyletic nature of *Mammillaria* as seen in our analyses.

Past circumscriptions of *Neolloydia* such as those of Hunt and Taylor (1986; 1990), have included the genera *Gymnocactus* Backeberg and *Turbinicarpus* (Backeberg) Buxbaum & Backeberg. Barthlott and Hunt (1993) noted that there were significant differences in the morphology between *N. conoidea* (type species) and other members of the genus, and suggested that a separate genus *Turbinicarpus* (presumably including *Gymnocactus*) may

be preferable. Hunt (1999) and Anderson (2001) accept a more narrow circumscription of *Neolloydia* by excluding from the genus those species that lack a tubercular groove and do not have axillary flowering areoles. Butterworth *et al.* (2002) supported the exclusion of members of *Turbinicarpus* from *Neolloydia*, clearly demonstrating that *Neolloydia conoidea* is phylogenetically positioned within their 'Mammilloid Clade' whose members have flowers that are positioned in an axillary position between the tubercles. The phylogeny presented in this paper further suggests that *Neolloydia conoidea* shows a closer relationship to *Mammillaria* species in Hunt's series *Ancistracanthae* and Lüthy's subgenus *Cochemiea* than to other species of *Mammillaria*.

Clade B. Clade 'B' and Clade 'T' of the parsimony and Bayesian analyses respectively are identical in their inclusivity and position (as a sister lineage to remaining members of *Mammillaria*). Hunt's (1981) treatment of *Mammillaria* distributes members of Clade 'B' among series *Sphacelatae* (*M. sphacelata* & *M. tonalensis*), *Ancistracanthae* (*M. zephyranthoides*), and *Stylothelae* (*M. oteroi*) all within subgenus *Mammillaria*, and subgenus *Oehmea* (*M. beneckei*). Lüthy's (1995; 2001) treatment of the genus, places these species into three groups – *Sphacelatae* (*M. sphacelata*, *M. tonalensis* and *M. oteroi*) in subgenus *Mammillaria*, series *Zephyranthoides* (*M. zephyranthoides*) in subgenus *Phellosperma*, and subgenus *Oehmea* (*M. beneckei*).

Mammillaria beneckei was recognized as a separate genus (*Oehmea*) by Buxbaum (1951a) based on the highly rugose nature of the seeds which allied the genus to his *Thelocactus* lineage. Hunt (1971) reunited *Oehmea* with *Mammillaria*, sinking it within subgenus *Dolichothele* of *Mammillaria*. Hunt later separated it from subgenus *Dolichothele* (Hunt, 1977a, 1981), but kept it as a subgenus in its own right due to various morphological differences from subgenus *Mammillaria*. The same stance on subgeneric recognition is also taken by Lüthy (1995; 2001) who accepts *Mammillaria beneckei* in subgenus *Oehmea*. Butterworth *et al.* (2002) noted that generic status for subgenus *Oehmea* is unwarranted and that Buxbaum's phylogenetic hypothesis of a close relationship between *Oehmea* and *Thelocactus* is incorrect,

and that *Oehmea* should be retained within *Mammillaria*. The phylogeny presented in this paper affirms those of Butterworth *et al.* and suggests that the inclusion of *Oehmea* within *Mammillaria* is justified.

When Buxbaum described the genus *Ebnerella* (Buxbaum, 1951c), he also described the subgenus *Archiebnerella* whose type species (*M. zephyranthoides*) formed the connecting (intermediate) group between *Neobesseya* and *Ebnerella*. Hunt subsequently sank *M. zephyranthoides* within his circumscription of series *Ancistracanthae* (Hunt, 1977a; 1981). Lüthy (1995; 2001) recognized *M. zephyranthoides* as being distinct from members of series *Ancistracanthae* and places the species together with *M. heidiae* Krainz in series *Archiebnerella*. Our phylogeny suggests that Hunt's placement of *Mammillaria zephyranthoides* into series *Ancistracanthae* is incorrect, although our sampling is insufficient to allow us to draw any conclusions regarding series *Archiebnerella*.

Clade C. Hunt's (1977b; 1981) circumscription of series *Stylothelae* included species possessing slender, soft-textured tubercles. The series was split into two groups by Hunt (1977b) – those species from the northwestern range of the series, with firm, blunt tubercles and acicular radial spines (*M. bombycina* Group) and those with a more southeastern distribution (*M. wildii* Group). Lüthy (1995) had a narrower circumscription of series *Stylothelae* than Hunt – a circumscription similar to Hunt's *M. wildii* Group. The other species were placed in series *Bombycinae* Lüthy. With the exclusion of *Mammillaria pottsii*, members of Clade 'C' correspond to Lüthy's circumscription of series *Stylothelae*. The inclusion of *Mammillaria pottsii* within this clade warrants further investigation. Hunt (1977b; 1986) and Pilbeam (1999) both allude to distinctive characteristics of this species, which Hunt placed within series *Leptocladodae*. Lüthy (1995) who did not include this species in his analyses, also placed *M. pottsii* in series *Leptocladodae*. The phylogeny presented in this paper suggests that *Mammillaria pottsii* is likely misplaced by both Hunt and Lüthy in series *Leptocladodae*.

Clade D. With the exception of *Mammillaria glassii*, members of Clade 'D' are treated by Hunt as members of series *Lasiacanthae* Hunt and *Proliferae* Hunt. In his description of series *Proliferae*, Hunt (1977b) cites that this group is distinct from members of series *Stylothelae* due to their straight central spines which intergrade with the radial spines rather than having two distinct series of spines. Hunt (1977b) further states that this series is linked to series *Lasiacanthae*, the latter lacking central spines altogether. *Mammillaria prolifera* and *M. picta* of Clade 'D' are included by Hunt (1981) in series *Proliferae*, and *M. carmenae, M. pectinifera* and *M. plumosa* are included in series *Lasiacanthae.* Lüthy (1995) accepted Hunt's placements of these species with the exception of *Mammillaria pectinifera* which he felt that along with *M. solisioides* deserved the recognition given them by Kuhn and Hofmann (1979) as series *Pectiniferae* Kuhn & Hoffmann.

Mammillaria glassii has been placed by Hunt (1984) and Lüthy (1995) into series *Stylothelae* and *Bombycinae* respectively. This species is distinguishable within series *Stylothelae* and *Bombycinae* by its spination with a single central spine that may be hooked or straight, and 6-8 subcentral spines that may be difficult to distinguish from the radial spines. For this reason, Hunt (1984) further suggested that *Mammillaria glassii* may form a link between series *Stylothelae* and *Proliferae.* Indeed, the phylogeny presented in this paper suggests that *Mammillaria glassii* has a greater affinity with members of series *Proliferae* and *Lasiacanthae* than it does to members of series *Bombycinae* and *Stylothelae.* Furthermore, our data suggest that series *Proliferae, Lasiacanthae* and *Pectiniferae* are very closely related.

Clade E. The topology of Clade 'E' forms a nested series of small clades, many of which lack strong statistical support. *Mammillaria decipiens, M. elongata* and *M. microhelia* seem to form a well-supported clade that forms a sister-lineage to remaining members of Clade 'E'. These species are placed within two series by both Hunt (1981) and Lüthy (1995). *Mammillaria decipiens* was used as the type species for Hunt's (1979) series *Decipientes* which he placed in subgenus *Dolichothele* due to its long tubercles, few spines and greenish fruits. Subsequently, Hunt (1981) removed series *Decipientes* from subgenus *Dolichothele* and al-

lied it with members of series *Leptocladodae* in subgenus *Hydrochylus*. Hunt further noted that the only known inter-series hybrid in *Mammillaria* occurred between series *Decipientes* and *Leptocladodae* in the cross between *M. decipiens* and *M. elongata*. Our phylogeny places members of series *Decipientes* and *Leptocladodae* in a single clade confirming Hunt's (1981) placement of these series alongside each other.

The clade containing *M. herrerae, M. humboldtii, M. candida* and *M. moelleriana* is supported by a bootstrap value of only one. Hunt (1981) grouped *M. herrerae* and *M. humboldtii* in series *Lasiacanthae* based mainly on the lack of central spines, numerous central spines, and globose, clustering habit. Lüthy (1995) separated these species from series *Lasiacanthae*, placing them in series *Herrerae* Lüthy within section *Krainzia* due to their seed and fruit morphology. The phylogeny presented in this paper supports the separation of these two species from series *Lasiacanthae* by Lüthy.

Described in 1838 by Scheidweiler, the treatment of *Mammillaria candida* has been a source of debate since Buxbaum (1951b) elevated the species to genus-level (*Mammilloydia*) based upon the verrucose seed testa. Hunt (1971) accepted that the seed of *M. candida* was unique among *Mammillaria* except that the seed differed due to its lack of intracellular pits. However, he felt that there was little else to separate it from *Mammillaria* and so adopted the treatment of Moran (1953) and accepted the subgenus *Mammilloydia* (Buxb.) Moran. Riha and Riha (1975) examined seeds of *Mammilloydia candida* from various sources and found that rather than having a verrucose testa (as stated by Buxbaum, 1951), seeds had a smooth testa. They concluded that Buxbaum's observations of the seed of *Mammilloydia candida* were inaccurate, even postulating that his material may have been contaminated. Furthermore, Riha and Riha also concluded that the lack of a pitted seed testa was not sufficient to warrant recognition of *Mammillaria candida* in its own subgenus or series, and suggested that the species would be better placed with members of Hunt's (1971) series *Lasiacanthae*. Hunt (1977a) contested the conclusions of Riha and Riha (1975) as superfluous, and continued to recognize the placement of *Mammillaria candida* within subgenus *Mammilloydia*. In 1986

and 1990, the working party of the International Organization for Succulent Plant Study (IOS) published preliminary findings on their search for a 'consensus' classification for the cactus family (Hunt and Taylor, 1986, 1990), in which *Mammillaria candida* was provisionally accepted within the genus *Mammillaria* in spite of unspecified differences which may warrant recognition as genus *Mammilloydia*. The International Cactaceae Systematics Group (formerly the IOS working party) concluded that generic-level recognition for *Mammilloydia candida* was justified (Hunt, 1999). Butterworth *et al.* (2002) concluded that recognition of the genus *Mammilloydia* would render *Mammillaria* paraphyletic. The phylogeny presented in this paper further supports their conclusion. Furthermore, our phylogeny groups *Mammillaria candida* with *M. herrerae* and *M. humboldtii*. Pilbeam (1999), comments on the resemblance of some forms of *M. humboldtii* to *M. herrerae*. More significantly however, past circumscriptions of *Mammillaria candida* such as those by Schumann (1898), Britton and Rose (1923), and Berger (1929) have sunk *Mammillaria humboldtii* within *Mammillaria candida*, whereas recent authorities such as Hunt (1984) and Pilbeam (1999) have dismissed similarities between these two species as superfluous. The phylogeny presented in this paper, suggests that *Mammillaria candida* should not be recognized at genus level (as *Mammilloydia*), and that this species is closely related to *Mammillaria humboldtii* and *M. herrerae*.

Also included within Clade 'E' is *Mammillaria longimamma*. Schumann (1898) viewed the elongate, soft tubercles of this species as sufficiently important to warrant its own subgenus – *Dolichothele* within *Mammillaria*. Britton and Rose (1923) elevated subgenus *Dolichothele* to genus level and it remained that way until Hunt (1971) sank it back into *Mammillaria* arguing that acceptance of *Dolichothele* at genus-level based only on one character or character-group was unjustified. Lüthy (1995) also accepts the sinking of *Dolichothele* into *Mammillaria* and (as Hunt) recognizes subgenus *Dolichothele*. Butterworth *et al.* (2002) concluded that Hunt and Lüthy were correct in treating *Mammillaria longimamma* as a member of *Mammillaria* and that this species was clearly not a separate genus. Our phylogeny further

supports this view, placing *M. longimamma* within the 'core' group of *Mammillaria* species. However, the phylogeny does not support recognition of *Dolichothele*, even at subgeneric level.

The sister group to *Mammillaria longimamma* includes *M. hernandezii* which is the only representative taxon from series *Longiflorae* Hunt. Members of this series are typically low-growing, cespitose plants with large flowers and black seeds. Hunt (1971) suggested that this group has affinities with members of series *Ancistracanthae*, placing them alongside each other in his classification, and that the disjunct distributions may be relictual or indicative that the two groups are not closely related. Lüthy (2001) also recognizes series *Longiflorae*, from Hunt in the placement of the group within *Mammillaria* in section *Krainzia* along with series *Herrerae* and series *Pectiniferae.*

With the exception of *M. lasiacantha* and *M. senilis*, the large clade that forms the sister group to *Mammillaria hernandezii* contains members treated within series *Stylothelae* by Hunt (1977b; 1981) who recognized a number of major species groups. Members of this clade formed Hunt's *M. bombycina* Group of series *Stylothelae* which Lüthy (1995) formally named as series *Bombycinae* Lüthy. The *M. bombycina* Group included the northern and western species of series *Stylothelae*, they tend to have larger, firmer and blunter tubercles, the radial spines are acicular and form in a single series.

Included within this clade is *M. senilis* whose distinct long-tubed, slightly zygomorphic flowers are bird pollinated. This species had been considered as distinct within the genus *Mammillaria.* Britton and Rose (1923) believed that morphological differences warranted treatment of this species in its own genus – *Mamillopsis* (Morren) Weber ex Britton & Rose. However, Hunt (1971) believed that *M. senilis* was not sufficently different from other members of *Mammillaria* to justify its separation into a separate genus and preferred to retain *Mamillopsis* at the rank of subgenus, a stance also taken by Lüthy (1995; 2001). The phylogeny presented in Figure 5-4 clearly indicates that recognition of *M. senilis* at subgenus-level would render subgenus *Mammillaria* polyphyletic. The placement of this species with *M.*

weingartiana appears unusual and warrants further investigation. However, it must be noted that the distribution of *M. senilis* in northern Mexico (Chihuahua, Durango and Jalisco) is sympatric with the distribtion of Clade 'E' members of series *Stylothelae.*

The inclusion of *Mammillaria lasiacantha* within this clade seems unusual. However, this species has been linked with members of this clade by a number of authors. Backeberg (1970) allied *Mammillaria gasseriana* Bödecker [= *M. stella-de-tacubaya sensu* Hunt (1984) and this paper] with *Mammillaria lasiacantha* in his series *Rectispinae* subseries *Amoenae.* Rogozinski and Appenzeller (1989 in Pilbeam, 1999) validated Backeberg's *M. egraria* which was invalidly published in 1951. However, they allied it with *Mammillaria stella-de-tacubaya* (in this paper). Hunt (1984; 1999) treats *M. egraria* as a subspecies of *Mammillaria lasiacantha* in series *Lasiacanthae.* The phylogeny presented in this paper, supports the link by Rogozinski and Appenzeller between *M. gasseriana* and *M. lasiacantha.*

CLADE F. Schumann (1898) divided Engelmann's subgenus *Eumamillaria* into two sections – *Hydrochylus* and *Galactochylus* for those species that had watery and milky sap respectively. In 1938, Backeberg described series *Subhydrochylus* to contain those species that possessed watery sap in the tubercles but milky sap in the stem core. Members of Clade 'F' correspond to sections *Mammillaria* (*Galactochylus*) and *Subhydrochylus* and are recognized by Hunt (1971; 1977b; 1977c; 1981; 1987). However, according to our phylogeny, series *Subhydrochylus* as currently circumscribed by Hunt is paraphyletic. *Mammillaria dioica* has been placed in series *Ancistracanthae* by both Hunt (1971; 1977a) and Lüthy (1995). The well-supported placement of *M. dioica* is anomalous and unexplainable morphologically, and warrants further investigation.

Except for the inclusion of a species pair consisting of *Mammillaria rhodantha* and *M. polythele*, series *Mammillaria* forms a monophyletic clade. Hunt (1971) places these two species in series *Heterochlorae* in section *Subhydrochylus.* However, he does note that series *Subhydrochylus* has morphological affinities with series *Ancistracanthae* and *Leptocladodae* (in

section *Hydrochylus*) while sharing other characteristics with series *Leucocephalae* and *Mammillaria* (section *Mammillaria*). Hunt (1977b) further concludes that series *Heterochlorae* (which includes *M. rhodantha* and *M. polythele*) needs an extensive revision.

Within Clade 'F', the clade containing *M. dixanthocentron, M. supertexta, M. huitzilopochtlii, M. albilanata, M. haageana* and *M. dioica* is well supported with 84% bootstrap and a decay value of 3 steps. With the exclusion of *M. dioica* from this clade (see above), members of this clade correspond with series *Supertextae* Hunt. Members of this series typically are shortly cylindrical to stoutly columnar, often clustering plants with small tubercles; small to very small flowers; central spines that are absent or, if present straight or curved; and numerous fine radial spines that obscure the stem. These morphological attributes are striking, and the series was also recognized by Lüthy (1995).

The well-supported clade containing *M. backebergiana, M. duoformis, M. magnifica, M. spinosissima* and *M. rekoi* have been placed within series *Polyacanthae* (Salm-Dyck) Schumann by both Hunt and Lüthy (Hunt, 1977b, 1981; Lüthy, 1995). Members of series *Polyacanthae* possess very small flowers, spines are numerous and differentiated into central spines, which may be hooked and numerous radial spines that rarely obscure the plant stem as in series *Stylothelae.*

The remaining sampled species of *Mammillaria* (with the exception of *M. rhodantha* and *M. polythele*) fall within Hunt's (1977c; 1981) and Lüthy's (1995) circumscriptions of section *Mammillaria* (*Galactochylus* Schumann). Members of this group are characterized by the presence of milky sap in stems and tubercles. The phylogeny presented in this paper shows that members of this section form a clade supported by a decay value of three steps. Within the Section *Mammillaria* Clade, the species *Mammillaria carnea, M. karwinskiana, M. polyedra, M. voburnensis* and *M. voburnensis* ssp.*eichlamii* form a polychotomy subtended by *M. mystax.* These species are recognized by Hunt (1977c; 1981) and by Lüthy (1995) within series *Polyedrae* (Pfeiffer) Schumann, and are characterized by their medium-sized flowers; few spines with little or no distinction between central and radial spines; and more-or-less

conspicuous axillary bristles (absent in *M. carnea*). The species subtending the 'Section *Mammillaria*' Clade – *M. melanocentra* ssp. *rubrograndis*, *M. grusonii* and *M. magnimamma* – are clearly distinguishable due to their distinct central and radial spines, and absent or inconspicuous axillary bristles.

The sister group to the clade containing series *Polyedrae* forms two clades held by three and two decay values respectively. The clade containing *M. peninsularis*, *M. petrophila* ssp. *baxteriana*, *M. lindsayi*, *M. polythele* and *M. rhodantha* contains members placed in series *Heterochlorae* and *Mammillaria* by Hunt (1977b; 1977c; 1981), and in series *Rhodanthae* and *Mammillaria* by Lüthy (1995). *Mammillaria rhodantha* and *M. polythele* form a species-pair in our phylogeny, and are referred by Lüthy (1995) to the new series *Rhodanthae* which he found to be phenetically distinct from members of series *Heterochlorae.* The other, closely related species to the *Rhodanthae* in our phylogeny are *M. peninsularis*, *M. petrophila* ssp. b*axteriana* and *M. lindsayi*, the former two species being found in southern Baja California, while the latter (*M. lindsayi*) is found across the Sea of Cortez in adjacent regions of Sinaloa and Chihuahua. The only other members of *Mammillaria* that occur in Baja California are from series *Ancistracanthae sensu* Hunt, clearly indicating independent migrations from mainland Mexico.

The remaining clade includes *M. cadereytensis*, *M. formosa*, *M. klissingiana*, *M. parkinsonii*, *M. bachmannii*, *M. geminispina* and *M. mammillaris.* With the exception of *Mammillaria mammillaris* (type species of *Mammillaria*), members of this clade have been referred to series *Leucocephalae* by Hunt (1971; 1977c; 1981) and Lüthy (1995).

Generic Circumscription of *Mammillaria*

The phylogenies presented in Butterworth *et al.* (2002) and this paper clearly show that as currently circumscribed, the genus *Mammillaria* is likely polyphyletic. Species within the genus *Coryphantha* and *Escobaria* are morphologically distinct from other members of *Mammillaria* due to the absence of the tubercular groove in members of *Mammillaria.* The

number of species in *Coryphantha* and *Escobaria* is 55 and 23 respectively. For this reason, firm conclusions regarding the polyphyly of *Mammillaria* due to the inclusion of members of these genera must be viewed with caution until more species are sampled. If increased sampling of species from *Coryphantha* and *Escobaria* reveal a monophyletic origin for these genera, then the obvious solution indicated by the phylogeny shown in Figure 5-4 is to restrict the genus *Mammillaria* to clades B through F.

Even if the genera *Coryphantha* and *Escobaria* form a separated clade from remaining members of clade 'A', the membership of clade 'A' is still problematic. *Neolloydia conoidea* and *Ortegocactus macdougallii* would need to be transferred from their respective genera. *Mammillaria halei, M. poselgeri* and *M. pondii* ssp. *setispina* are currently placed by both Hunt and Lüthy in subgenus *Cochemiea* Brandegee, which itself was validly elevated by Walton in 1899 to the rank of genus. Thus the *Mammillaria* members of clade 'A', *Neolloydia conoidea* and *Ortegocactus macdougallii* would be transferable to genus *Cochemiea* (Brandegee) Walton.

In summary, the phylogeny presented in this paper suggests that as currently circumscribed, the genus *Mammillaria* is polyphyletic on a number of levels with members of series *Cochemiea* and *Ancistracanthae* probably being referable to genus *Cochemiea*. Within the 'core' group of *Mammillaria*, past taxonomic classifications (chiefly Hunt and Lüthy) have had limited success in identifying 'natural', phylogenetic groups, and to some extent, have been thwarted by morphological convergence in a genus that likely contains numerous 'micro' taxa.

With regard to a more detailed infrageneric classification of *Mammillaria*, the amount of uncertainty due to poorly supported clades within the core group of *Mammillaria* urges caution. Future investigations are ongoing with the intention of increasing the depth of sampling within the genera *Coryphantha* and *Escobaria* as well as filling-in sampling gaps within *Mammillaria*. It is also imperative to add more molecular data such as other 'fast' evolving chloroplast and nuclear markers to further add support at branch tips, and slightly slower markers to add robustness to major branches towards the root of the phylogeny. Once a well-

supported phylogeny has been produced, assessments of morphology can be utilized along with phylogenetic information to yield a reliable infrageneric classification within *Mammillaria*.

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CHAPTER 6

A Localized Loss of the Chloroplast *rpl16* Intron from *Mammillaria* Series *Stylothelae*

A short paper to be submitted to the journal Heredity

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INTRODUCTION

Of the structural rearrangements that occur within the chloroplast genome, those that are considered relatively rare have been used as a source of robust phylogenetic data as reviewed by Downie and Palmer (1992). Doyle *et al.* (1995) found that in Leguminosae, the *rpl2* intron has been lost at least four times, and concluded that the loss of an intron provides more reliable phylogenetic data than the loss of a gene. The loss of entire introns has been documented in various plant taxa (Downie *et al.*, 1991); in grasses (Katayama and Ogihara, 1993); in Geraniaceae, Goodeniaceae and Plumbaginaceae (Campagna and Downie, 1998); and in cacti (Wallace and Cota, 1996).

In this paper, we report the loss of the *rpl16* intron from various members of *Mammillaria* Series *Stylothelae* Pfeiffer ex Schumann. We also discuss the implications of the intron loss on the phylogenetics, biogeography and classification of these cacti.

MATERIALS AND METHODS

Taxonomic Sampling

A total of 115 members of *Mammillaria* were sampled as part of a comprehensive phylogenetic study (Butterworth and Wallace, in prep.). Of these taxa, 33 are classified in Series *Stylothelae sensu* Hunt (1987) and are listed in Table 6-1.

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[4] Major Professor.

Table 6-1. Taxa sampled for the rpl16 intron and psbA–trnH IGS study. ISC = Ada Hayden Herbarium, Iowa State University.

Taxon	Source/Voucher	Intron	Source of Evidence
Mammillaria anniana Glass & Foster	W.A. Fitz Maurice 2193	–	Sequence
Mammillaria bocasana Poselger	W.A. Fitz Maurice 1916	–	Sequence
Mammillaria bombycina Quehl	W.A. Fitz Maurice 1821	+	Sequence
Mammillaria brachytrichion Luethy	W.A. Fitz Maurice 2358	+	Sequence
Mammillaria crinita De Candolle	W.A. Fitz Maurice 2153	–	Sequence
Mammillaria crinita De Candolle	W.A. Fitz Maurice 2346	–	Gel Photo
Mammillaria crinita ssp. *leucantha* (Boedeker) Hunt	W.A. Fitz Maurice 2199	–	Sequence
Mammillaria duwei Rogozinski & Appenzeller	W.A. Fitz Maurice 1641	–	Sequence
Mammillaria erythrosperma Boedeker	W.A. Fitz Maurice 1766	–	Sequence
Mammillaria fittkaui Glass & Foster	W.A. Fitz Maurice 2107A	–	Sequence
Mammillaria gasseriana Boedeker	W.A. Fitz Maurice 2289	+	Sequence
Mammillaria glassii Foster	HNT 60162—ISC	+	Sequence
Mammillaria jaliscana (Britton & Rose) Boedeker	W.A. Fitz Maurice 1817	+	Sequence
Mammillaria limonensis Reppenhagen	W.A. Fitz Maurice 2222	–	Sequence
Mammillaria marcosii W.A. & B. Fitz Maurice & Glass	W.A. Fitz Maurice 2364	–	Sequence
Mammillaria mathilde Glass & Foster	W.A. Fitz Maurice 1647	–	Gel Photo
Mammillaria mercadensis Patoni	W.A. Fitz Maurice 2332	+	Sequence
Mammillaria mercadensis Patoni	W.A. Fitz Maurice 2344	+	Sequence
Mammillaria moelleriana Boedeker	W.A. Fitz Maurice 2336	+	Sequence
Mammillaria nana Backeberg ex Mottram	W.A. Fitz Maurice 1980	–	Sequence
Mammillaria nazasensis (Glass & Foster) Reppenhagen	W.A. Fitz Maurice 2323	+	Sequence
Mammillaria pennispinosa Krainz	W.A. Fitz Maurice 2273	+	Sequence
Mammillaria perezdelarosae Bravo & Scheinvar	W.A. Fitz Maurice 1644	+	Sequence
Mammillaria rettigiana Boedeker	W.A. Fitz Maurice 2091	+	Sequence
Mammillaria schwarzii Shurly	W.A. Fitz Maurice 1687B	–	Sequence
Mammillaria sinistrohamata Boedeker	W.A. Fitz Maurice 2316	+	Sequence
Mammillaria stella-de-tacubaya Heese	W.A. Fitz Maurice 2322	+	Sequence
Mammillaria tezontle Fitz Maurice	W.A. Fitz Maurice 1983	–	Sequence
Mammillaria weingartiana Boedeker	W.A. Fitz Maurice 1544	+	Sequence
Mammillaria wildii Dietrich	W.A. Fitz Maurice 2190	–	Sequence
Mammillaria zacatecasensis Shurly	W.A. Fitz Maurice 2020	+	Sequence
Mammillaria zeilmanniana Boedeker	W.A. Fitz Maurice 1764	–	Gel Photo

DNA Extraction and Purification

Extractions of total genomic DNA of representative taxa were carried out using one of three methods:

1. Modified organelle pellet method suitable for mucilaginous material. DNA was extracted from despined, green plant material according to previously published methods (Butterworth *et al.*, 2002; Wallace, 1995; Wallace and Cota, 1996), and the DNA pellet was resuspended in 1ml of TE.

2. Nucleon Phytopure™ plant and fungal kit for 1g samples (Amersham Life Science). Extracted DNA was resuspended in 1ml TE and stored at -20°C.

3. DNEasy Plant Mini kit (Qiagen). Approximately 90mg of green plant material was used for each extraction. The manufacturer's protocol was followed with the exception that the DNA was eluted in 50μL of sterile distilled water.

Amplification and Sequencing

Double-stranded amplification of the target sequences was done using the Polymerase Chain Reaction (PCR) conducted in a MJ Research PTC-100 thermal cycler. Primers used for amplification and sequencing were those of Campagna and Downie (1998) – Rpl16F (5' TTTCCTTTCGAAAAGCAATG 3') and Rpl16R (5' TCTTCCTCTATGTTGTTTACG 3'). The structural organization of the *rpl16* gene and flanking regions is shown in Figure 6-1.

PCR amplifications were carried out in 100 μL reactions which included 10 μL of 10X buffer, 5 μL of 25 mmol/L magnesium chloride solution, 8 μL of 25 mmol of an equimolar dNTP solution, 20 pmol of each primer, 0.5 μL of Taq polymerase, and 2 μL of unquantified DNA template. The following temperature cycling parameters gave sufficient amplification of the *rpl16* intron: an initial melting at 95°C for 5 min followed by 24 cycles of the following protocol: 1) 95°C melt for 2 min; 2) 50°C annealing for 1 min; 3) ramp temperature increase of 15°C at 0.125°C per sec; 4) 65°C extension for 4 min. A final extension step at 65°C for 10 min completed the PCR amplification.

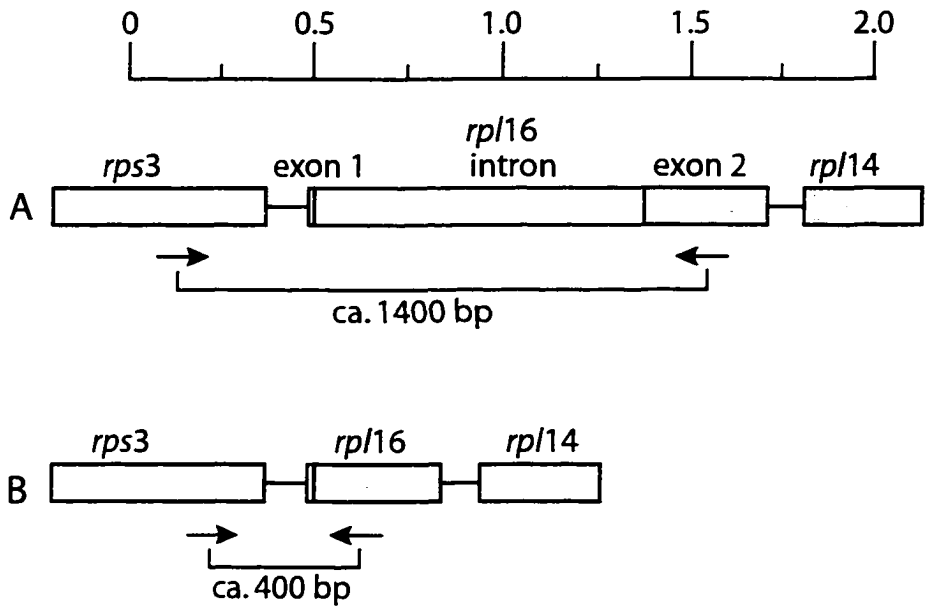

Figure 6-1. Organization of the *rpl*16 gene and adjacent genes. The shaded boxes indicate coding regions, the *rpl*16 intron is indicated by the open box. The scale shows units in kilobases. The arrows show the position of the forward and reverse primers. (A) In the majority of cacti species sampled, the *rpl*16 gene is comprised of two exons separated by an intron of approximately 1,400 bp. (B) In members of *Mammillaria* that lack the *rpl*16 intron, the PCR product is approximately 400 bp. Figure redrawn from Campagna and Downie (1998).

PCR products were spun in a vacuum centrifuge to reduce their volumes to approximately 10 μL, run into a 1.5% TAE agarose gel. The amplicon bands were excised from the gel and cleaned using one of the following two methods: 1) Geneclean II kit (Bio 101) according to the manufacturer's instructions. Elution from the glassmilk pellet was achieved in 10 μL sterile distilled water followed by a second elution in 5 μL sterile distilled water; 2) QIAquick Gel Extraction kit (Quiagen) according to the manufacturer's instructions. Elution was in 30μL sterile distilled water followed by a second elution in 20 μL sterile distilled water, the purified product was further concentrated in a vacuum centrifuge to a final volume of approximately 10 μL. Purified PCR products from both protocols were quantified using agarose electrophoresis using a 1% gel in TAE buffer. 1 μL of concentrated, purified PCR product was run into the gel alongside a quantity standard that consisted of two lanes containing 10 μL and 5 μL respectively of ϕX174-*HAEIII* (Invitrogen) at a concentration of 25 μg/ml.

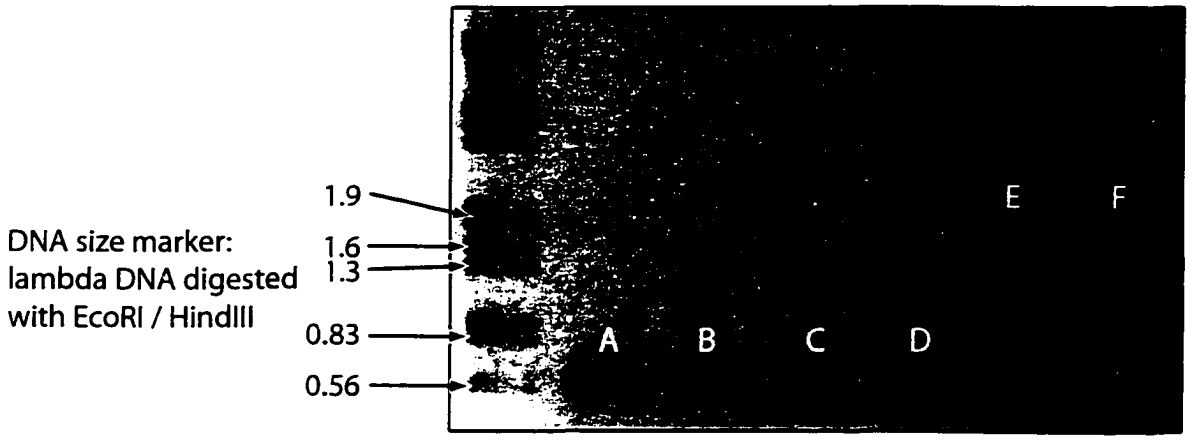

Figure 6-2. Gel photo from PCR amplifications of the *rpl*16 gene and flanking regions. Taxa shown are: A. *Mammillaria wildii* (WAF 2190); B. *M. zeilmanniana* (WAF 1764); C. *M. crinita* (WAF 2346); D. *M. mathilde* (WAF 1647); E. *M. pennispinosa* (WAF 2273); F. *M. gasseriana* (WAF 2289). DNA fragment sizes are in kilobases.

Sequence data were obtained in chain-termination reactions using the ABI Prism Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). Approximately 200 ng of purified PCR products were used for sequencing. For most of the sequencing reactions, 1:4 dilutions of the BigDye solution gave acceptable reads, however for some amplicons, dilutions of 1:1 BigDye solution were required to yield acceptable DNA sequences. Electrophoresis and automated sequence reading were undertaken at the Iowa State University Protein Facility using Perkin Elmer / Applied Biosystems automatic sequencing units (ABI Prism 377).

Results

Of the taxa sampled, 16 showed the deletion of the *rpl*16 intron (Table 6-1). For those taxa that were not sequenced, the intron loss is clearly observed in gel photos from the original PCR amplification (Figure 6-2). For those "intronless" taxa that were sequenced using the primers of Campagna and Downie (1998), the sequences are shown in Figure 6-3 and clearly demonstrate perfect excision and deletion of the intron at the splice sites established by Michel *et al.* (1989) for group II introns. The remaining exons of the *rpl*16 gene form a single, uninterrupted gene. The mechanism involved in such precise splicing and subsequent

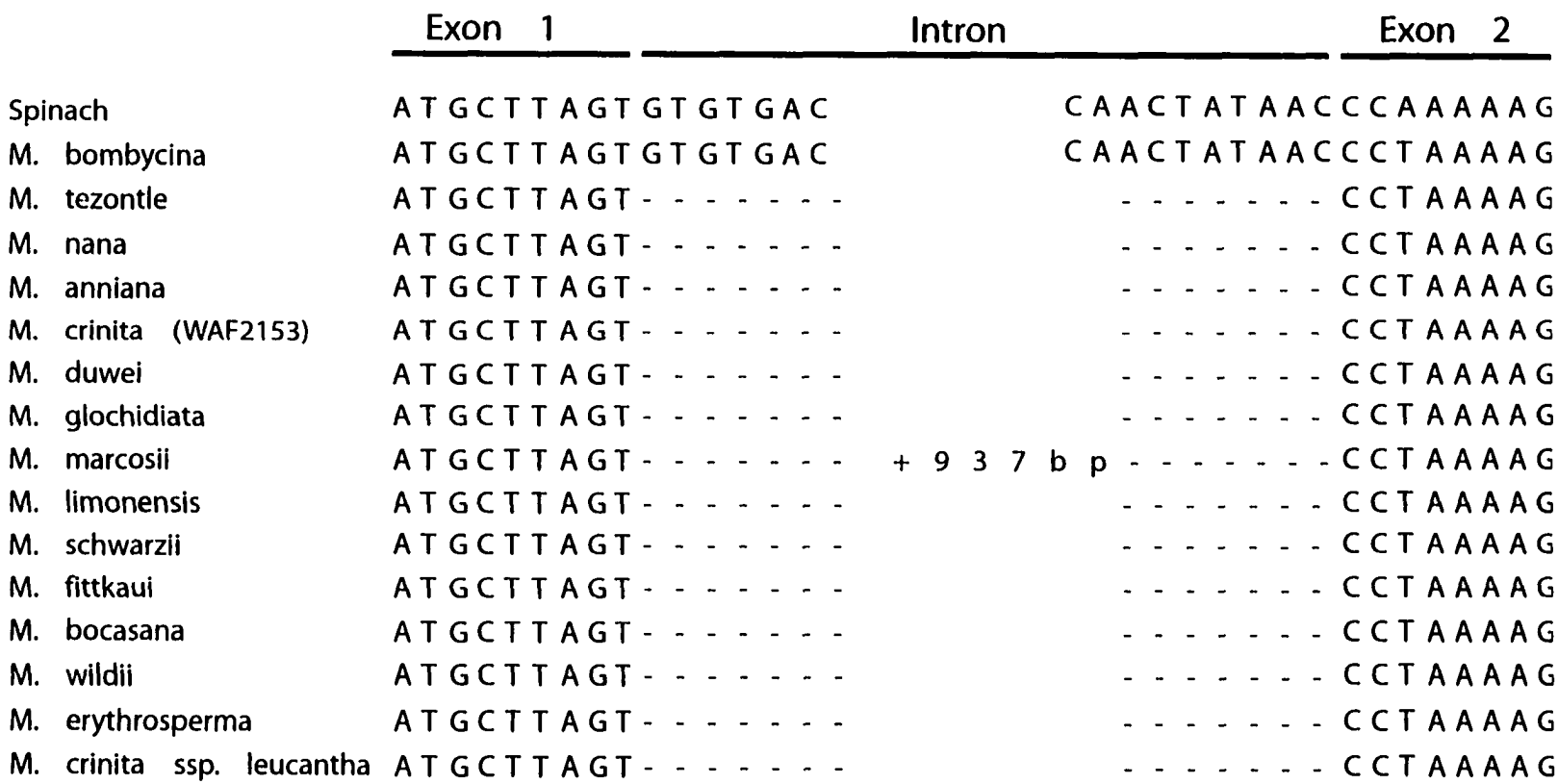

Figure 6-3. Sequence alignment of the *rpl*16 intron. Members of *Mammillaria* series *Stylothelae* possessing the intron are aligned against those lacking the intron, which has been precisely excised as the intron splice sites. The reference sequence for spinach is taken from Schmitz-Linneweber *et al.* (2001).

loss of an entire intron is not fully known, although a number of researchers (Downie *et al.*, 1991; Hiratsuka *et al.*, 1989) speculate that the spliced RNA (lacking the intron) may undergo reverse transcription and reintegration into the chloroplast genome.

Discussion

Taxonomic Groupings

Recent research using chloroplast DNA sequence data (Butterworth and Wallace, in prep.) has clearly demonstrated that there are at least two main divisions within *Mammillaria* series *Stylothelae* as circumscribed by Hunt (1981). These divisions correspond roughly to Lüthy's circumscriptions of series *Bombycinae* and *Stylothelae.* All taxa of *Mammillaria* sampled to date that possess the *rpl*16 intron deletion belong in series *Stylothelae sensu* Lüthy and forms a robust synapomorphy for that circumscription. Fitz Maurice and Fitz Maurice (inedit) take the broad circumscription of series *Stylothelae* that Hunt recognizes. However, they divide series *Stylothelae* into four species groups, of which the *Crinita* Group includes all the sampled taxa that lack the *rpl*16 intron.

Morphology

There are a number of clear morphological differences separating those taxa within series *Stylothelae* that possess the *rpl*16 intron from those that have lost the intron. However, none of the morphological differences are mutually exclusive in either the intron-present or intron-absent groups.

Flowering Time. All members of the *Crinita* Group sampled in this paper with the exception of *Mammillaria fittkaui* flower between March and August. The other members of series *Stylothelae* have a flowering period that runs from November to April. Although there are occurrences of flowering times overlapping, it seems plausible that the disjunct flowering periods may allow a number of the species to occur sympatrically over parts of their distributions.

Flower and Fruit Insertion. In members of the *Crinita* Group, the flowers and subsequent fruit are only superficially inserted into the tubercle axils. This contrasts with members of the other species groups in series *Stylothelae* whose members possess flowers and fruit that are firmly inserted into the tubercle axils.

Axillary Bristles. All sampled members of the *Crinita* Group possess bristles in the tubercle axils. With the exception of *Mammillaria perezdelarosae* and *M. bombycina* which are placed in the *Bombycina* Group and *M. glassii* which is placed in the *Glassii* Group (Fitz Maurice and Fitz Maurice, inedit), intron-possessing members of series *Stylothelae* tend to lack axillary bristles.

Seed Testa Pitting. The seed testas of all members of series *Stylothelae* have pits. Sampled members of the *Crinita* Group with the exception of *M. erythrosperma* have coarsely pitted seeds, whereas those in the *Mercadensis* and *Glassii* groups have finely pitted seed testas.

Biogeography

The sampled taxa of *Mammillaria* series *Stylothelae sensu* Hunt are distributed throughout central Mexico (see Figure 6-4). Members of the *Crinita* Group are typically found east of 102°W in the states of Guanajuato, Hidalgo, Jalisco, Queretero, San Luis Potosí, and Tamaulipas while those members of the *Mercadensis* Group are distributed mainly west of 102°W, in the states of Coahuila, Durango, Jalisco, Nuevo Leon, and Zacatecas. Sampled members from the *Bombycina* Group are found towards the southern end of the distribution of Series *Stylothelae*, straddling the 102°W meridian in the states of Aguascalientes and Jalisco.

In summary, the apparent rarity of precise intron excisions from the genome, combined with the limited distribution (in relation to *Mammillaria*) and morphological similarities clearly indicate that the deletion of the *rpl*16 intron in these cacti is the result of a single event. Thus, the deletion of the *rpl*16 intron provides valuable data in the classification and

circumscription of *Mammillaria* Series *Stylothelae sensu* Hunt (1981; 1987), strongly suggesting that the narrower circumscription taken by Lüthy (1995) may be more appropriate.

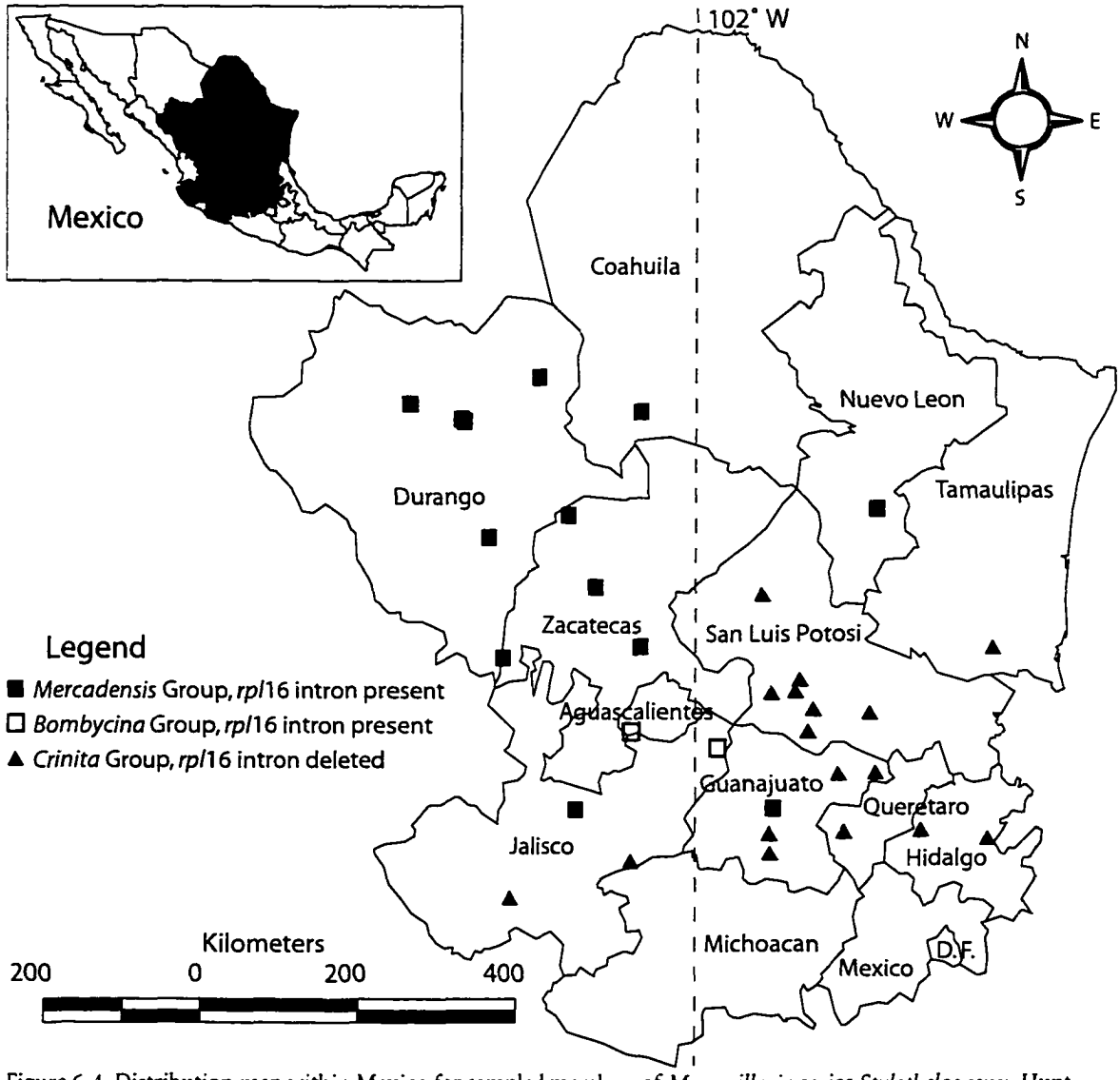

Figure 6-4. Distribution map within Mexico for sampled members of *Mammillaria* series *Stylothelae sensu* Hunt (1987). D.F. = Mexico City.

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Chapter 7

General Conclusions

Systematics of Tribe Cacteae

The use of sequence data from the *rpl*16 intron resulted in a phylogeny for the tribe that has a pectinate topology. The earliest divergence in the Cacteae split *Aztekium* and *Geohintonia* from the remaining members of the tribe. There also appears to be an evolutionary transition in Tribe Cacteae from stem ribs to stem tubercles, although there appear to be a number of reversals. Another transition that seems to be secondary to the presence of stem tubercles is the position of the flowering areole, which in derived members of the tribe (*Ariocarpus* and the "Mammilloid" Clade) are distinct from the spine-bearing areoles, and located in the tubercle axils.

The "Mammilloid" Clade appears to be united by the presence of two synapomorphies – tuberculate stem morphology and the presence of dimorphic areoles. Buxbaum (1951b) places members of this clade into a number of lines ('lineas') – Thelocacti, Neobesseyae and Coryphanthae. Buxbaum (1958) places members of this clade into subtribe Thelocactinae Buxb., Ferocactinae Buxb., and Coryphanthanae B. & R. emend Buxb. The phylogeny of the tribe Cacteae presented in Chapter 4 suggests the reassignment and restriction of subtribe Coryphanthinae to include only those members included in the "Mammilloid" Clade.

One of the research goals of the study of Tribe Cacteae was to resolve issues regarding the selection of a suitable outgroup for the genus *Mammillaria*. Past taxonomic treatments of both Tribe Cacteae and *Mammillaria* (detailed in Chapters 2 & 3) strongly urged caution towards the choice of *Coryphantha* and *Escobaria* as outgroup members. Indeed, the phylogeny from Chapter 4 revealed the paraphyletic nature of *Mammillaria* and suggested that a more rational choice of outgroup taxa for *Mammillaria* research would be taxa that occur more basal to the "Mammilloid" Clade. For this reason, members of *Stenocactus* and *Ferocactus* were chosen as outgroup representatives for the *Mammillaria* study presented in Chapter 5.

Mammillaria Systematics

Generic Circumscription

Extensive sampling from members of *Mammillaria*, coupled with the use of two chloroplast markers yielded a reasonably well-resolved phylogeny for the genus. Further to the findings of the study on Tribe Cacteae, the genus *Mammillaria* was found to be clearly polyphyletic as presently circumscribed. A major division in members currently recognized as *Mammillaria* placed in series *Ancistracanthae* and subgenus *Cochemiea sensu* Hunt (1981) in a clade containing sampled taxa from *Coryphantha, Escobaria, Neolloydia* and *Pelecyphora*. *Mammillaria* species of this clade must certainly be questionable in terms of their inclusion within *Mammillaria*. However, until a better understanding of relationships of these species with *Coryphantha, Escobaria, Neolloydia* and *Pelecyphora* is achieved, it would be imprudent to make any taxonomic changes to these taxa at present.

Hunt and Lüthy Reconciled

The classification of *Mammillaria* produced by Hunt (1971; 1977a; 1977b; 1977c; 1981; 1984; 1986; 1987) was the result of a thorough familiarity with the genus rather than a scientific approach to classification. Lüthy (1995; 2001) applied a phenetic approach to the infrageneric classification of *Mammillaria* and produced a classification that mainly differs from that of Hunt in the treatment of the what Lüthy terms the "primitive" members of *Mammillaria*. A comparison of the treatments of Hunt and Lüthy is shown in Chapter 3, Figure 3-3. By taking the phylogeny of *Mammillaria* from Chapter 5 and excluding taxa that are noncontentious in the Hunt and Lüthy classifications, a comparison between the two classification systems for *Mammillaria* is possible (see Figure 7-1, 7-2, 7-3).

Subgenera of *Mammillaria*

Until the most recent CITES Cactaceae Checklist (Hunt, 1999), David Hunt recognized six subgenera in *Mammillaria*. The treatment of *M. candida* within *Mammillaria* as a subgenus was a compromise situation for Hunt, who felt that the verrucose seed testa in this species justified its recognition at genus level. Lüthy also treated this species at the rank of genus, consequently not analyzing it as part of his studies. The phylogeny presented in Chapter 5 strongly suggests that recognition of *Mammilloydia* as a distinct genus is not warranted (see Figure 7-1). Furthermore, the position of *Mammillaria candida* within the 'core' group of *Mammillaria* strongly suggests that placement in its own subgenus is also not supported by molecular data.

Both Hunt and Lüthy agree in the recognition of subgenus *Oehmea*, even though Lüthy did not sample this taxon. Buxbaum (1951a) recognized this species (*M. beneckei*) as a distinct genus *Oehmea* due to the rugose seed testa. However, the phylogeny presented in Chapter 5 clearly indicates that the recognition of a distinct subgenus for *Mammillaria beneckei* by Hunt and Lüthy is unjustified as it would render subgenus *Mammillaria sensu* Hunt and subgenus *Phellosperma sensu* Lüthy paraphyletic (see Figure 7-1).

Restricted to a single species (*M. senilis*), Hunt's subgenus *Mamillopsis* is treated at sectional rank by Lüthy. This species forms small clumps of globular to cylindric stems; flowers are long-tubed, red or vermillion, and hummingbird pollinated; and at least two of the central spines are hooked. These characteristics have been used to ally this species with members of *Cochemiea*. However, the phylogeny in Figure 7-1 clearly separates these groups and recognition of *Mamillopsis* at the rank of subgenus would make subgenus *Mammillaria* polyphyletic.

Subgenus *Dolichothelae* has identical circumscriptions in the infrageneric classifications of *Mammillaria* by both Hunt and Lüthy. The main factor that distinguishes this taxon from other members of *Mammillaria* is the presence of elongate, soft tubercles. The phylogeny presented in Figure 7-1 confidently places subgenus *Oehmea* within the clade comprised

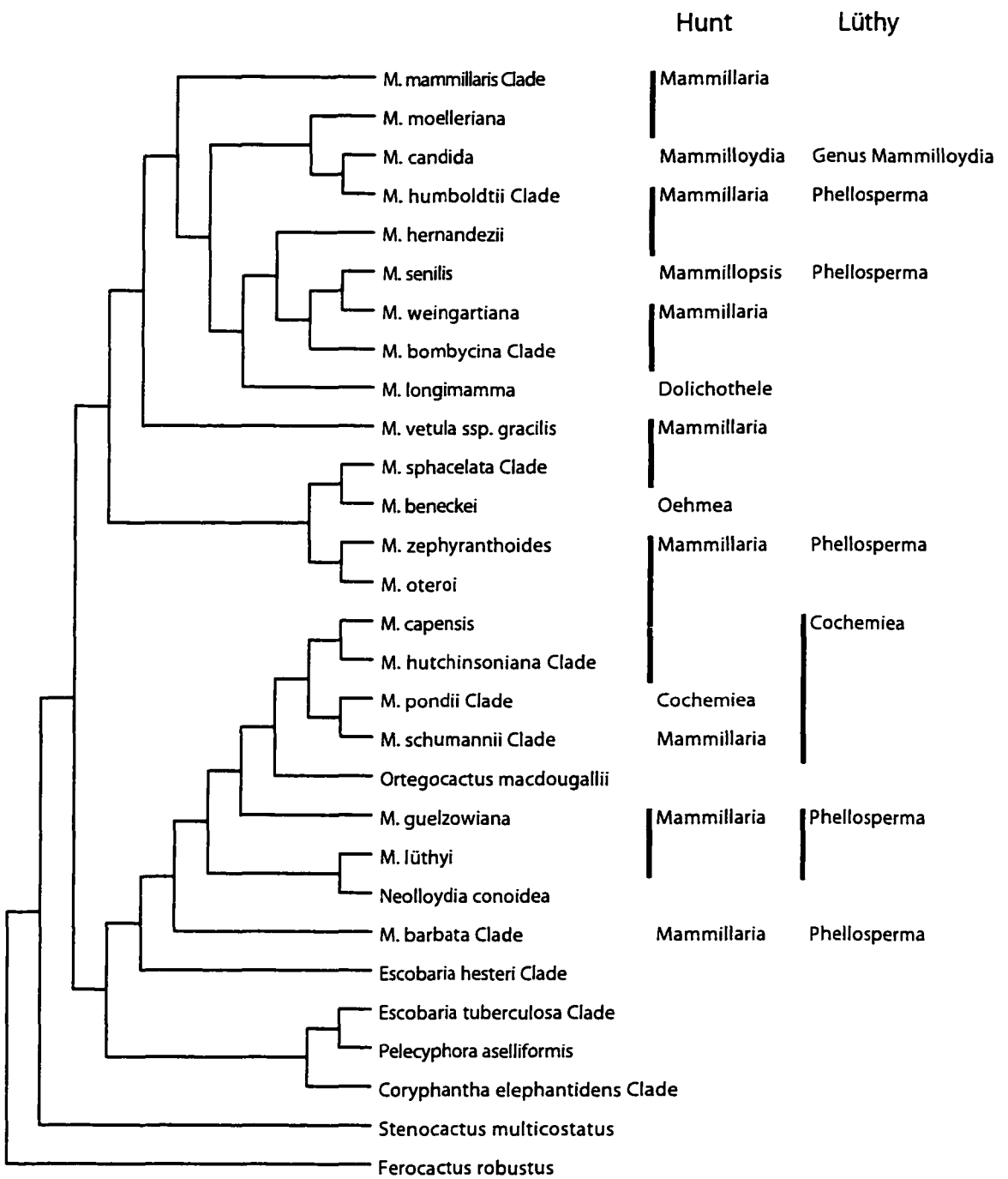

Figure 7-1. The phylogeny of *Mammillaria* and the infrageneric classification of Hunt (1987) showing subgenera. Lüthy's (1995, 2001) classification is shown alongside where it differs from that of Hunt. The phylogeny is based on the majority-rule consensus cladogram from Chapter 5 except that clades whose memberships are unanimous for a specific subgenus are collapsed. Not shown in this figure is *Mammillaria dioica* (treated as subgenus *Mammillaria* by Hunt and subgenus *Cochemiea* Lüthy) and *M. discolor* (treated as subgenus *Mammillaria* by Hunt and Lüthy) which are placed within the *M. mammillaris* Clade and the *M. hutchinsoniana* Clade respectively in the phylogeny from Chapter 5.

of members of subgenus *Mammillaria*, rendering the latter paraphyletic. Thus recognition of subgenus *Oehmea* is not supported.

In his infrageneric classification of *Mammillaria* Hunt restricts membership of subgenus *Cochemiea* to those species with elongated cylindrical stems and narrowly tubular flowers with bilateral symmetry that are hummingbird pollinated. Lüthy applies a wider circumscription, preferring to include those species that have hypodermal druses, and a very short zone of stamen insertion in the floral tube. Acceptance of subgenus *Cochemiea sensu* Hunt or Lüthy renders subgenus *Mammillaria* polyphyletic. However, Lüthy's circumscription according to the phylogeny in Figure 7-1 makes a monophyletic group that forms a sister-taxon to *Ortegocactus*.

The circumscriptions of subgenus *Mammillaria* by both Hunt and Lüthy are polyphyletic due to the inclusion of the other subgenera. Lüthy adopted a more narrow circumscription of subgenus *Mammillaria* than Hunt did by segregating those species with a very solid receptacle, long insertion zone of the stamens, thick-walled epidermis, and globular to short fruits into subgenus *Phellosperma*. This separation results in a circumscription of subgenus *Mammillaria* that (although paraphyletic) corresponds much better to the 'core' *Mammillaria* in the phylogeny presented in Chapter 5.

Sections of *Mammillaria*

Schumann (1898) divided *Mammillaria* subgenus *Eumamillaria* Schum. into two sections (*Hydrochylus* and *Galactochylus*) based upon the presence or absence of milky latex in the plant body. Hunt further divided section *Hydrochylus* into section *Subhydrochylus* and *Mammillaria* (= *Galactochylus*). According to the phylogeny shown in Figure 7-2, all of Hunt's sections are at most paraphyletic.

Lüthy's sectional divisions of *Mammillaria* have narrower circumscriptions than those of Hunt. Furthermore, Lüthy also treats section *Hydrochylus* and *Subhydrochylus* as synonyms

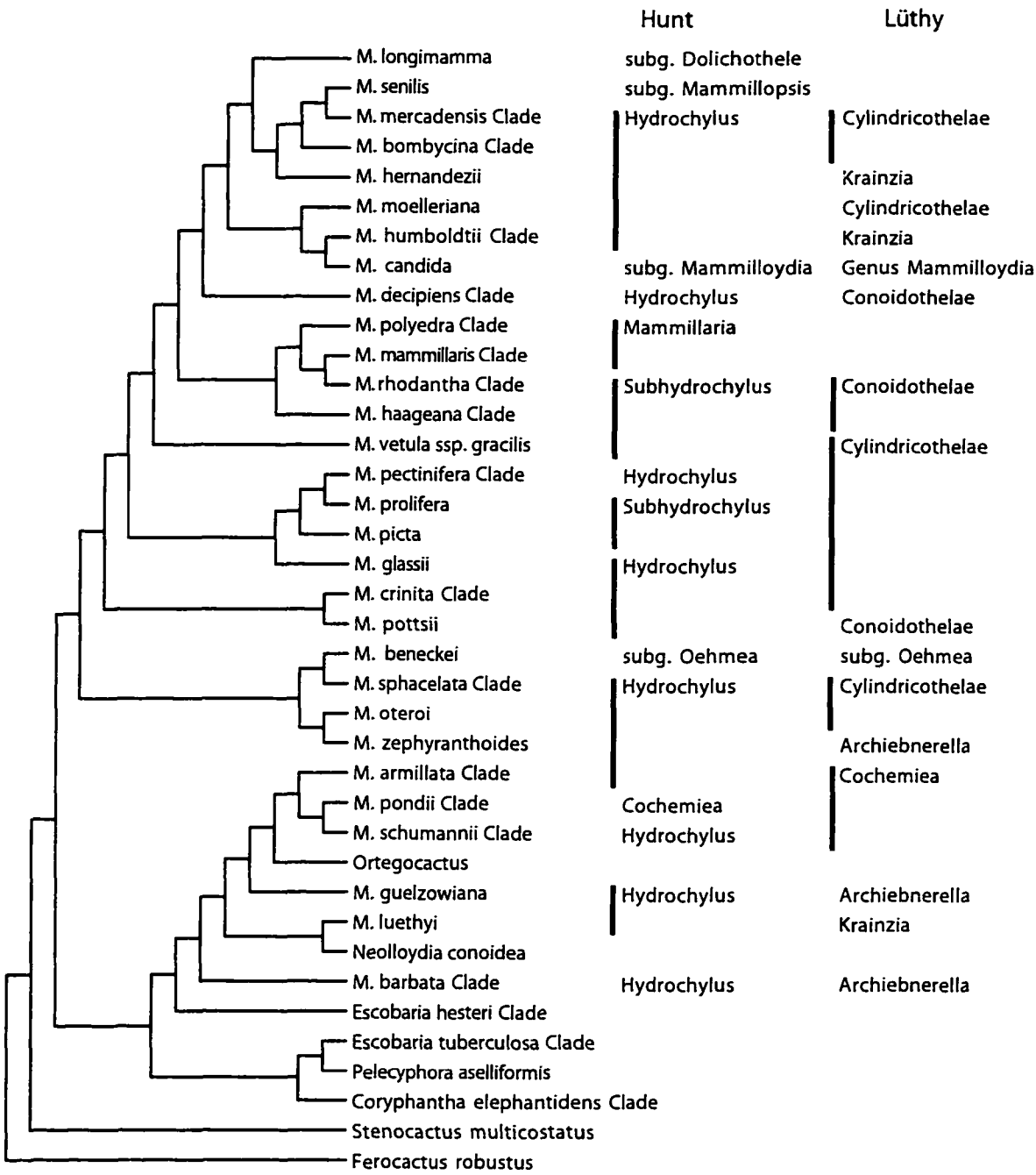

Figure 7-2. The phylogeny of *Mammillaria* and the infrageneric classification of Hunt (1987) showing sections. Lüthy's (1995, 2001) classification is shown alongside where it differs from that of Hunt. The phylogeny is based on the majority-rule consensus cladogram from Chapter 5 except that clades whose memberships are unanimous for a specific section are collapsed. Not shown in this figure is *Mammillaria dioica* (treated as section *Hydrochylus* by Hunt and section *Cochemiea* by Lüthy) and *M. discolor* (treated as section *Subhydrochylus* by Hunt and section *Conoidothelae* by Lüthy) which are placed within the *M. haageana* Clade and the *M. hutchinsoniana* Clade respectively in the phylogeny from Chapter 5.

of Lemaire's earlier *Cylindricothelae* and *Conoidothelae* respectively. However, in spite of the narrower circumscriptions, all sections recognized by Lüthy are paraphyletic according to the phylogeny in Figure 7-2.

Series of *Mammillaria*

On the whole, the infrageneric classifications of Hunt and Lüthy agree quite well at the level of series, the major differences lying in those members of the 'primitive' *Mammillaria*.

SERIES *ANCISTRACANTHAE* SCHUMANN. Hunt's circumscription of series *Ancistracanthae* is clearly polyphyletic according to the phylogeny presented in Figure 7-3 due to the placement of series *Cochemiea*, *M. luethyi* (which Hunt places in series *Lasiacanthae*), and the genera *Ortegocactus* and *Neolloydia*. Lüthy recognizes series *Bartschella* and includes within it those species that Hunt treats as close relatives, but in series *Ancistracanthae*. Lüthy also recognizes series *Phellosperma* as distinct from Hunt's *Ancistracanthae*. However, in spite of these differences, Lüthy's circumscriptions of series *Ancistracanthae*, *Bartschella* and *Phellosperma* are shown to be paraphyletic.

SERIES *STYLOTHELAE* (PFEIFFER) SCHUMANN. The members of series *Stylothelae sensu* Hunt was divided into two series by Lüthy based mainly upon the presence or absence of hooked central spines. Those members with hooked central spines remained in series *Stylothelae* while those lacking hooked central spines, were recognized within series *Bombycinae*. The phylogeny summarized in Figure 7-3, does indeed support the distinct separation of these series, as does the deletion of the *rpl*16 intron. Lüthy's circumscription of series *Stylothelae* is monophyletic according to the phylogeny in Figure 7-3, although series *Bombycinae* is polyphyletic.

SERIES *PROLIFERAE* HUNT. Both Hunt and Lüthy circumscribe series *Proliferae* in a similar manner. This group of species, found mainly in NE Mexico, includes *Mammillaria prolifera* which has an extensive range from southern Texas, the West Indies and also reported from Colombia. According to the phylogeny presented in Figure 7.3, series *Proliferae* is poly-

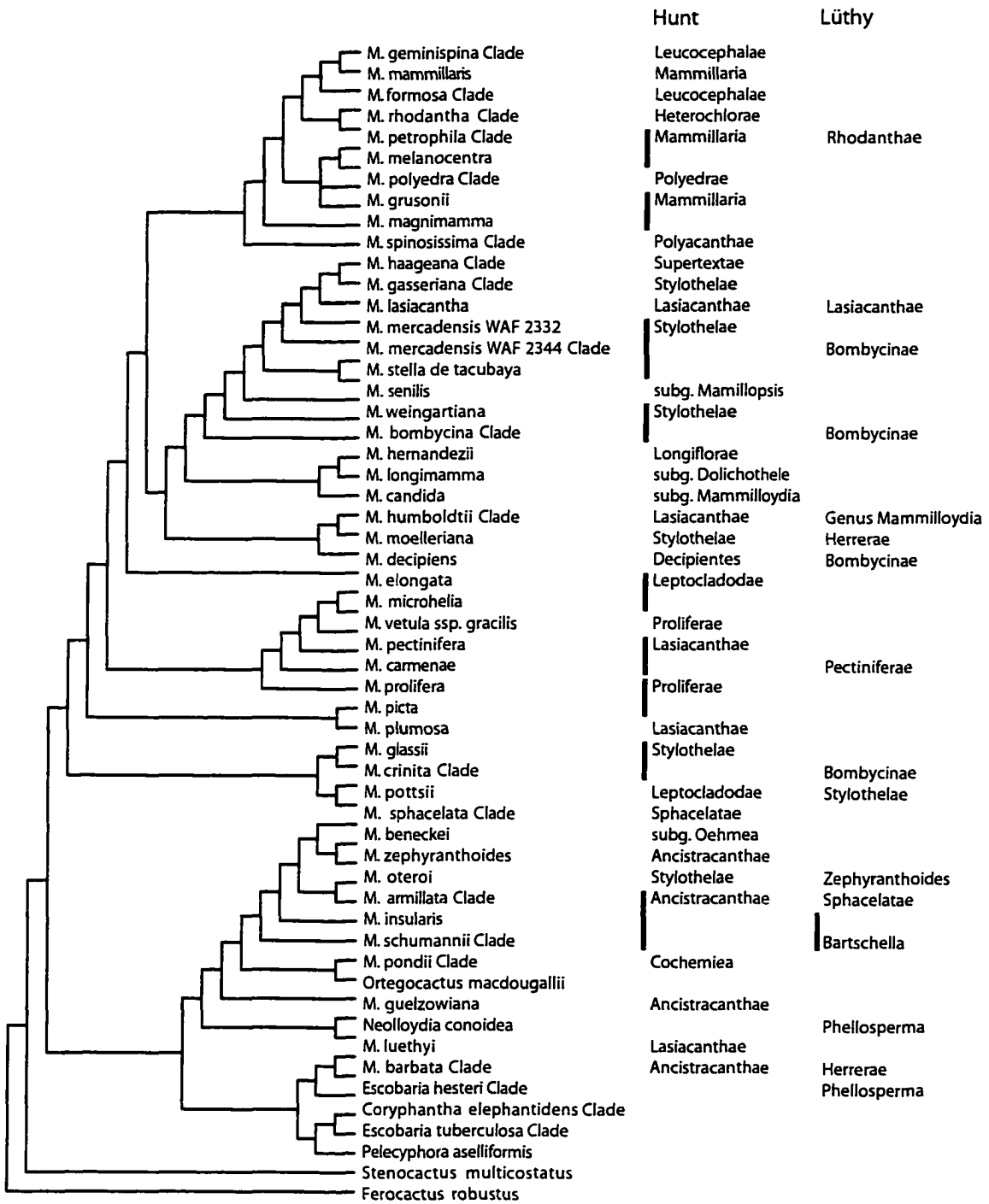

Figure 7-3. The phylogeny of *Mammillaria* and the infrageneric classification of Hunt (1987) showing series. Lüthy's (1995, 2001) classification is shown alongside where it differs from that of Hunt. The phylogeny is based on the majority-rule consensus cladogram from Chapter 5 except that clades whose memberships are unanimous for a specific series are collapsed. subg. = subgenus. Not shown in this figure is *Mammillaria dioica* (treated as series *Ancistracanthae* by both Hunt and Lüthy) and *M. discolor* (treated as series *Heterochlorae* by Hunt and Lüthy) which are placed within the *M. haageana* Clade and *M. armillata* Clade respectively in the phylogeny from Chapter 5.

phyletic due to the placement of *M. glassii* (series *Stylothelae/Bombycinae*), *M. plumosa* (series *Lasiacanthae*) and *M. vetula*.

Series *Lasiacanthae* Hunt. Members of series *Lasiacanthae* typically lack central spines and possess numerous spines on globular plants. Hunt takes a broad circumscription of the series, which Lüthy divided treated as three series – *Lasiacanthae, Pectiniferae* and *Herrerae*. The phylogeny in Figure 7.3 clearly demonstrates that as circumscribed by Hunt, series *Lasiacanthae* is polyphyletic. Even the narrower circumscriptions proposed by Lüthy fare little better, with series *Lasiacanthae sensu* Lüthy being paraphyletic due to the placement of *M. lasiacantha* and *M. plumosa*. Series *Herrerae* was also found to be paraphyletic due to the placements of *M. humboldtii* Clade and *M. lüthyi*. Sampling of series *Pectiniferae* was limited to only *M. pectinifera* thus assessments of monophyly of this series cannot be made.

Series *Sphacelatae* Hunt. Series *Sphacelatae sensu* Hunt is monophyletic according to the phylogeny in Figure 7.3. This series is restricted to a small number of species from southern Central Mexico. Lüthy also included *M. oteroi* within series *Sphacelatae*. Hunt had placed this species within series *Stylothelae* although he suggested that he had misgivings about placing *M. oteroi* with other species found much further north. However, in Figure 7.3, it is clear that the taxonomic placements of subgenus *Ohemea*, and *M. zephyranthoides* render Lüthy's circumscription of series *Sphacelatae* paraphyletic.

Series *Leptocladodae* (Lemaire) Schumann. Hunt and Lüthy have similar circumscriptions of series *Leptocladodae*. The phylogeny on Figure 7.3 indicates that this series is paraphyletic due to the disjunct placement of *M. pottsii*, which is recognized by Hunt as a distinct species within the series. The remaining members of series *Leptocladodae* in Figure 7.3 (*M. elongata* and *M. microhelia*) form a paraphyletic assemblage due to the inclusion of *M. decipiens* which both authors treat in series *Decipientes*.

Series *Heterochlorae* (Salm-Dyck) Schumann. Series *Heterochlorae sensu* Hunt uses a broader concept than that of Lüthy, who separates series *Rhodanthae* to include those species

that Hunt allied together in his *M. rhodantha* Group. The sampling of taxa in Chapter 5 is insufficient to fully resolve issues of monophyly or paraphyly for this series.

Series *Polyacanthae* (Salm-Dyck) Schumann. The phylogeny shown in Chapter 5 (Figure 5.4) shows a well-supported clade of species that includes *M. spinosissima*, which is treated as the *M. spinosissima* Clade in Figure 7.3. Hunt and Lüthy both recognize this group as a distinct series – *Polyacanthae* which is named due to the numerous, long radial spines that are characteristic of the type species of the series (*M. spinosissima*). The data in Chapter 5 clearly shows that this series is monophyletic.

Series *Supertextae* Hunt. Series *Supertextae* is recognized by both Hunt and Lüthy, and according to the phylogeny presented in Chapter 5 (and Figure 7.3) is monophyletic (*M. haageana* Clade).

Series *Leucocephalae* (Lemaire) Schumann. As currently circumscribed by Hunt and Lüthy, series *Leucoocephalae* is paraphyletic due to the placement of *Mammillaria mammillaris* (series *Mammillaria* and type species for the genus). Members of this series typically have conspicuous axillary bristles, which may be somewhat obscured by numerous radial spines in some species; the plant bodies often undergo dichotomous branching to form quite large clumps.

Series *Mammillaria*. As circumscribed by Hunt and Lüthy, this series is polyphyletic according to the phylogeny in Figure 7.3. *M. mammillaris* is shown to be more closely related to members of series *Leucocephalae* than to other members of series *Mammillaria* (*M. petrophila* Clade, *M. melanocentra*, *M. grusonii* and *M. magnimamma*).

Series *Polyedrae* (Pfeiffer) Schumann. The species sampled in Chapter 5 that belong to series *Polyedrae* form a monophyletic clade. Members of this series are typically from southern Mexico (*M. voburnensis* extends into Guatemala) they may also have axillary bristles and can undergo dichotomous branching, indicating morphological similarities with members of series *Leucocephalae*.

HUNT AND LÜTHY RECOMPILED

Given the implied complexities of the generic circumscription of *Mammillaria* presented in Chapter 5 (Figure 5.4 – reproduced in this chapter as Figure 7.4), any form of infrageneric classification of members of Clade 'A' is unwise at this time. However, those sampled *Mammillaria* species belonging in clade 'B' through 'F' quite clearly belong in a single taxon either comprising the genus *Mammillaria* or as subgenus *Mammillaria* based upon a final choice of membership for members of Clade 'A'. Within the 'core' *Mammillaria* (clades 'B' through 'F') a number of clades appear to lend themselves to treatment at the rank of series.

SERIES *SPHACELATAE* HUNT in Cact. Succ. J. G.B. 39(3): 73, 1977.

Type species = *M. sphacelata* Mart.

Synonyms: genus *Leptocladodia* Lemaire ex Buxbaum; subgenus *Leptocladodae* Lemaire; genus *Leptocladia* Buxbaum; subgenus *Leptocladodia* Lemaire ex Bravo; genus *Oehmea* Buxbaum; subgenus *Oehmea* (Buxbaum) Hunt; series *Zephyranthoides* Kuhn & Hoffmann.

Included species: *M. sphacelata* Martius; *M. tonalensis* Hunt; *M. beneckei* Ehrenberg; *M. oteroi* Glass & Foster; *M. zephyranthoides* Scheidweiller.

SERIES *STYLOTHELAE* PFEIFFER EX SCHUMANN in Gesambesch. der Kakteen: 516, 1898.

Type species = *M. wildii* Dietrich.

Synonyms: *Stylothelae* Pfeiffer; section *Crinitae* Salm-Dyck; section *Hydrochylus* Schumann; genus *Ebnerella* Buxbaum; subgenus *Euebnerella* Buxbaum; section *Euebnerella* Buxbaum; genus *Chilita* Orcutt.

Included species: *M. wildii* Dietrich; *M. fittkaui* Glass & Foster; *M. mathilde* Glass & Foster; *M. zeilmanniana* Boedecker; *M. anniana* Glass & Foster; *M. bocasana* Poselger; *M. crinita* De Candolle; *M. erythrosperma* Boedecker; *M. schwarzii* Shurly; *M. limonensis* Rep-

penhagen; *M. marcosii* Fitz Maurice, Fitz Maurice and Glass; *M. nana* Backeberg ex Mottram; *M. duwei* Rogozinski & Appenzeller; *M. tezontle* Fitz Maurice.

UNAMED SERIES

Type species: *M. pottsii* Scheer ex Salm-Dyck.

Included species: *M. pottsii* Scheer ex Salm-Dyck.

This species, is placed as a sister taxon to all members of *Mammillaria* series *Stylothelae*, as described above, in a well-supported clade (see Figure 7.4). However, all sampled members of series *Stylothelae* are lacking the entire *rpl*16 intron (see Chapter 6). *Mammillaria pottsii* possesses the *rpl*16 intron. *Mammillaria pottsii* is characterized by low-growing stems that branch at the base; tubercles ovate to obtuse with somewhat woolly axils (lacking bristles as in series *Stylothelae*); radial spines numerous (to 45), 2.5 to 3cm long, white; central spines from 5 to 11, more robust than the radial spines, recurving, up to 12mm in length, brown to blue-black, not hooked (*Stylothelae* possess hooked central spines); flowers up to 15mm long and wide, preianth red-brown; fruit red; seed dark brown to black.

SERIES *PROLIFERAE* HUNT in Cact. Succ. J. G.B. 39(3): 73, 1977.

Type species: *M. prolifera* (Miller) Haworth.

Synonyms: subgenus *Rectochilita* Buxbaum; section *Rectochilita* Buxbaum; genus *Solisia* Britton & Rose; subgenus *Solisia* (Britton & Rose) Moran; series *Pectiniferae* Kuhn & Hoffmann.

Included species: *M. prolifera* (Miller) Haworth; *M. carmenae* Casteneda & Nunez; *M. pectinifera* Weber; *M. picta* Meinshausen; *M. plumosa* Weber; *M. glassii* Foster.

SERIES *LEPTOCLADODAE* (LEMAIRE) SCHUMANN in Gesambesch. der Kakteen: 515, 1898.

Type species: *M. elongata* De Candolle.

Synonyms: *Leptocladodae* Lemaire (unspecified infrageneric rank); genus *Leptocladia* Buxbaum; genus *Leptocladodia* Lemaire ex Buxbaum; subgenus *Leptocladodia* Lemaire ex Bravo; series *Decipientes* Hunt.

Included species: *M. elongata* De Candolle; *M. decipiens* Scheidweiler; *M. microhelia* Werdermann.

SERIES *CANDIDAE* SCHUMANN in Gesambesch. der Kakteen: 515, 1898.

Type species: *M. candida* Scheidweiler.

Synonyms: genus *Mammilloydia* Buxbaum; subgenus *Mammilloydia* (Buxbaum) Moran; series *Herrerae* Lüthy.

Included species: *M. candida* Scheidweiler; *M. herrerae* Werdermann; *M. humboldtii* Ehrenberg.

SERIES *BOMBYCINAE* LÜTHY in Taxon. Unter. Gattung *Mammillaria*: 154, 1995.

Type species: *M. bombycina* Quehl.

Synonyms: subgenus *Euancistracantha* Buxbaum; section *Euancistracantha* Buxbaum.

Included species: *M. bombycina* Quehl; *M. perezdelarosae* Bravo & Scheinvar.

UNAMED SERIES

Included species: *M. mercadensis* Patoni; *M. brachytrachion* Lüthy; *M. nazasensis* (Glass & Foster) Reppenhagen; *M. pennispinosa* Krainz; *M. sinistrohamata* Bödecker; *M. jaliscana* (Britton & Rose) Bödecker; *M. zacatecasensis* Shurly; *M. rettigiana* Bödecker; *M. gasseriana* Bödecker; *M. lasiacantha* Engelmann; *M. stella-de-tacubaya* Heese.

SERIES *SUPERTEXTAE* HUNT in Cact. Succ. J. G.B. 39(4): 98, 1977.

Type species: *M. supertexta* Martius ex Pfeiffer.

Included species: *M. supertexta* Martius ex Pfeiffer; *M. dixanthocentron* Backeberg ex Mottram; *M. huitzilophchtlii* Hunt; *M. albilanata* Backeberg; *M. haageana* Pfeiffer.

SERIES *POLYACANTHAE* (SALM-DYCK) SCHUMANN in Gesambesch. der Kakteen: 516, 1898.

Type species: *M. spinosissima* Lemaire.

Synonyms: section *Polyacanthae* Salm-Dyck.

Included species: *M. spinosissima* Lemaire; *M. backebergiana* Buchenau; *M. duoformis* Craig & Dawson; *M. magnifica* Buchenau; *M. rekoi* (Britton & Rose) Vaupel.

SERIES *POLYEDRAE* PFEIFFER EX SCHUMANN in Gesambesch. der Kakteen: 563, 1898.

Type species: *M. polyedra* Martius.

Synonyms: *Polyedrae* Pfeiffer (unspecified infrageneric rank).

Included species: *M. polyedra* Martius; *M. carnea* Zuccarini ex Pfeiffer; *M. karwinskiana* Martius; *M. voburnensis* Scheer; *M. mystax* Martius.

TAXA INSERTAE SEDIS: Increased sampling within series *Rhodanthae, Heterochlorae, Leucocephalae* and *Mammillaria* will help resolve issues with infrageneric classifications in a large number of the unplaced taxa. Other placements should become apparent through the use of additional DNA markers. Unplaced *Mammillaria* taxa yet to be sufficiently studied are: *M. cadereytensis* Craig: *M. formosa* Galeotti ex Scheidweiler; *M. klissingiana* Bödecker; *M. parkinsonii* Ehrenberg; *M. bachmanii* Bödecker ex Berger; *M. geminispina* Haworth; *M. mammillaris* (L.) Karsten; *M. peninsularis* (Britton & Rose) Orcutt; *M. petrophila* Brandegee; *M. lindsayi* Craig; *M. polythele* Martius; *M. rhodantha* Link & Otto; *M. melanocentra* Poselger; *M. grusonii* Runge; *M. magnimamma* Haworth; *M. dioica* Brandegee; *M. senilis* Loddiges ex Salm-Dyck;

M. weingartiana Bödecker; *M. hernandezii* Glass & Foster; *M. longimamma* De Candolle; *M. moelleriana* Bödecker; *M. vetula* Martius.

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